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Abstract Book

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1 Sexual adaptation across evolution: the neural basis of female sexual motivation

Eya Wolfson[1], Shachaf Shapira[1,2], Rizwanul Haque[1], Mattia Morandi[2], Elena Fidel[3], Shifra Ben-Dor[3], Gil Stelzer[3], Neta Regev-Rudzki[2], Meital Oren-Suissa[11]Brain Sciences, Weizmann Institute of Science, [2]Biomolecular Sciences, Weizmann Institute of Science, [3]Life Sciences Core Facilities, Weizmann Institute of Science

Throughout the course of evolution, species are constantly adapting to increase survivability and fitness according to their specific needs and constraints. But how are these processes integrated into neural networks to efficiently promote desired behaviors that benefit the individual? We address this key question in evolutionary neuroscience by exploring a striking example of adaptive evolution of female sexual behavior. The existence of hermaphroditic species alongside females within the *Caenorhabditis* genus provides a unique opportunity to compare their sexual behavior. We identified and characterized a novel set of female mating behavioral features. Strikingly, we discovered that while hermaphrodites are indifferent to males, females of *C. afra, C. japonica*, *C. brenneri* and *C. inopinata* exhibit a pronounced sex drive towards males, actively engaging in and initiating mating. Importantly, we found that under induced reproductive pressure across generations, sperm-deficient *C. elegans* hermaphrodites (*fog-2* mutants) switch to female-like behaviors, suggesting the existence of a suppressed neuronal mechanism. We compared the transcriptomes of 1st and 20th generation *fog-2* pseudofemales (F0, F20), and *C. afra* females, and identified downregulation of neuropeptides as a key modulator of female mating behavior. Importantly, we were able to evoke female sexual behaviors in hermaphrodites by knocking-down *nlp-18, nlp-2* and *ins-24* neuropeptides, which were significantly downregulated in F20 animals. Complementarily, we were able to inhibit sexual drive in *C. afra* females by overexpressing *nlp-18* in their nervous system. Despite the lack of any obvious anatomical changes in their sensory neurons, females exhibit a clear attraction to male-conditioned media (MCM), which is absent in hermaphrodites. Finally, we have established a method to measure neuronal activity in *C. afra*, and identified a unique activity pattern of ASE-like neurons in response to MCM in females and F20 pseudofemales. Our data suggest that silencing ASE neurons in both *C. afra* females and F20 pseudofemales inhibits their response to males, further proving sexual attraction to be mediated by ASE and its downstream partners. Together, our work offers an opportunity to explore the origins of female sexual attraction, a topic that is often overlooked, how it is encoded in neural networks, and the intricate ways by which the nervous system adapts to accommodate evolutionary pressures.

2 wormFISH: A whole-animal *In Situ* spatial transcriptomics technique for *C. elegans*

Wenhan Chang, Bob HorvitzBiology, HHMI/MIT

C. elegans was the one of the first organism to have transgenic GFP reporters and used in single-molecule Fluorescence *In Situ* Hybridization (smFISH), and has since been an invaluable model for studying spatial-temporal gene expression at cellular resolution. Its simple and completely described anatomy and cell lineage combined with Its diverse tissue and cell types make *C. elegans* an excellent animal for studies of cell diversity and differentiation. However, existing methods are either limited by low throughput, restricting analysis to only a few genes – e.g., fluorescent reporters, *in situ* hybridization, and antibody staining - or disrupt spatial context during sample preparation, as with both bulk and single-cell RNA-Seq. These constraints prevent the interrogation of transcriptomic states at single-cell resolution within an individual worm, restricting analyses to either a small set of genes or pooled populations from different individuals. We have developed a novel *in situ* spatial transcriptomic technique, called wormFISH, for multiplexed gene expression profiling of intact worms. wormFISH achieves single-cell resolution and can simultaneously profile over 100 genes. We also have developed an analytical pipeline for the quantitative and holistic characterization of the resulting data at 3D single-cell resolution. We validated wormFISH by comparing its results with results using established cell-specific markers and CRISPR knock-in reporters. We confirmed the specificity of wormFISH using CRISPR knock-outs. Our data show that wormFISH accurately recapitulates endogenous gene expression patterns in intact animals. wormFISH can be applied to worms with different genetic backgrounds or exposed to different environmental conditions, revealing transcriptomic differences at the single-cell level. Given the diverse cell and tissue types in *C. elegans*, wormFISH provides a powerful spatial transcriptomics method for studying how individual cells adapt to genetic and environmental perturbations, enabling new quantitative insights into single-cell responses in intact animals.

3 Nematode Hunters: CUREing Intracellular Infections

Jessica N SowaBiology, West Chester University of Pennsylvania

Nematode Hunters is a community science effort that leverages elementary school outreach, course-based undergraduate research (CURE), and traditional lab-based research to discover novel intracellular pathogens of wild nematodes. Each fall participating 4th grade classrooms conduct a week-long classroom module where students learn about nematodes, collect samples from their local environment, identify samples containing wild nematodes, and ultimately submit the nematodes to WCU to be screened for intracellular infections. These samples are screened for intracellular infections by 16 students enrolled in the Nematode Hunters CURE course, which runs each fall at WCU in conjunction with the outreach program. Students in the CURE develop research skills by assuming responsibility for a set of wild nematode samples. Over the course of the semester they conduct co-culture assays with infection reporter *C. elegans* to test for infection transmission, and design and perform their own experiments to determine the nature of any infections identified. Survey data is collected from both 4th grade and undergraduate participants to evaluate whether participation in the program affects student perceptions of themselves as scientists and interest in science careers.

Using this approach we have evaluated over 500 wild nematode isolates, identifying more than 200 potential intracellular infections. Our approach has identified several new variants of the Orsay virus, a number of novel strains of microsporidia, and a new intestinal fungal infection. Data from pre and post surveys indicates that participation in Nematode Hunters leads to both an increased knowledge of how science is conducted and recognition that they have participated in the scientific process for 4th graders in the program, and analysis of undergraduate student outcomes from the CURE is underway.

4 Caenorhabditis Genetics Center

Aric L Daul, Julie Knott, Kat Piloto, Adam Grazzini, Amorette Vang, Ann E RougvieGCD, Univ of Minnesota

The Caenorhabditis Genetics Center (CGC) promotes C. elegans research by curating important genetically characterized nematode stocks and distributing them to researchers and science educators throughout the world. The CGC is housed at the University of Minnesota and is supported by the National Institutes of Health - Office of Research Infrastructure Programs (NIH-ORIP) and user fees. We have shipped ~54,000 strains to ~2,000 different labs over the last two years. We strive to have at least one null allele and one functional endogenously-tagged allele of every gene. If you have generated such strains that are not represented in the collection, please contact us about making a donation. We are also interested in useful chromosomal rearrangements, duplications, deficiencies, select multiple-mutant stocks, disease models, and genetic tool strains for various applications such as inducible gene expression. A searchable list of our nearly 27,000 strains, including information about each stock, is accessible through the CGC website (cgc.umn.edu) and WormBase. Orders must be placed on-line through our website, using credit cards for payments whenever possible. We provide yearly reports to the NIH with statistics that reflect our services to the worm community. A key tracked parameter is the number of published papers that acknowledge the CGC for providing strains. Please help us maintain our funding by acknowledging the CGC in your publications!

5 The Kinetochore Protein, KNL-1, regulates the actin cytoskeleton to control dendrite branching

Henrique Alves Domingos, Dhanya Cheerambathur School of Biological Sciences, University of Edinburgh

Precise control of dendritic branching in individual neurons is central to building the complex structure of the nervous system. Despite significant advances in understanding the signalling pathways that shape dendrites, the intrinsic mechanisms that shape their branches remain largely unclear. We had previously shown that the kinetochore protein KNL-1 and its associated KMN (Knl1/Mis12/Ndc80 complex) network partners, typically known for their role in chromosome-microtubule coupling during mitosis, control dendrite branching in the *C. elegans* mechanosensory PVD neuron (PMID:39625434). Kinetochore proteins were shown to limit excessive dendritic branching and promote contact-dependent repulsion, ensuring proper sensory function and preventing premature neurodegeneration of the PVD neuron.

KNL-1 is a highly conserved scaffold protein that recruits both the signalling proteins and microtubule binding elements to the kinetochore. Unexpectedly, KNL-1 was found to regulate F-actin dynamics in the PVD neuron to establish appropriate dendritic architecture. Its N-terminal region, which harbors signaling activity, directly initiates F-actin assembly at the plasma membrane, suggesting that regulation of actin assembly is intrinsic to the kinetochore proteins. Here, we reveal that KNL-1 generates dynamic actin filament structures by recruiting the Arp2/3 complex and its upstream regulators to the plasma membrane to shape the dendritic arborization pattern. Our findings suggest that a crosstalk between kinetochore proteins and the actin regulatory network—mediated by Rho family proteins downstream of extrinsic cues—dictates proper dendrite patterning in PVD neurons.

These results highlight a novel postmitotic role for the neuronal kinetochores in shaping the developing nervous system through actin cytoskeleton regulation, offering new insights into the mechanisms underlying dendrite architecture and neuronal connectivity.

6 The functions of *unc-43/CaMKII* and *pkc-2/PKC* in Wnt-dependent neurite pruning

Menghao Lu, Jeffery Lin, Ilham Hakim, Kota Mizumoto University of British Columbia

During development, neurons remove excessive neuronal processes, or neurites, via a mechanism called developmental neurite pruning. Previously, we showed that LIN-44/Wnt instructs neurite pruning of the postembryonic cholinergic motor neuron, PDB, at the L2 stage by recruiting its receptor LIN-17/Frizzled (Fz) to the pruning neurites (Lu and Mizumoto, 2019). We found that the temperature-sensitive mutant of the IP3 receptor, *itr-1(sa73)*, exhibits the PDB pruning defects, while the gain-of-function mutation (*R582Q*) in *itr-1(gof)* suppresses the pruning defect of *lin-44(allele)* mutant, suggesting that LIN-44 regulates PDB neurite pruning through IP3R-dependent calcium signaling.

We then looked for calcium-dependent protein kinases as potential effectors of Wnt-dependent neurite pruning, and found that loss-of-function (lof) mutant of *unc-43/*calmodulin-dependent protein kinase II (CaMKII), *unc-43 (n498n1186),* exhibits ectopic neurites in PDB at the L4 stage, suggesting that *unc-43* is required for neurite pruning. *unc-43(n498n1186)* does not enhance the pruning defect of *lin-44(n1792lof),* while the *unc-43(n498)* gain-of-function (gof) allele suppresses the pruning defect of *lin-44(n1792).* Additionally, expressing *unc-43* cDNA in PDB rescues the pruning defect of *unc-43(n498n1186)* mutant. These data suggest that *unc-43* functions downstream of Wnt, and acts cell-autonomously in PDB to regulate neurite pruning. The penetrance of pruning defects of *unc-43(allele)* is less severe compared to *lin-44(allele)*, suggesting that there is additional factor that act in parallel to *unc-43* in Wnt-dependent neurite pruning. We found that, while *pkc-2(ok328lof)* single mutant exhibits a mild pruning defect in PDB, the *unc-43(n498n1186); pkc-2(ok328)* double mutants exhibits a severe neurite pruning defect comparable to *lin-44(n1792)* mutant. Moreover, *unc-43(n498n1186)* and *pkc-2(ok328)* do not enhance the pruning defect of *lin-44(n1792)*, suggesting that *lin-44, unc-43,* and *pkc-2* function in the same genetic pathway.

Lastly, we observed the accumulation of early endosome marker, RAB-5, in the pruning neurite in a PKC-2-dependent but UNC-43-independent manner. Consistently, genetic epistatic analysis suggested that the clathrin-dependent endocytic pathway acts in the same genetic pathway as *pkc-2* but in parallel to *unc-43,* suggesting PKC-2 and UNC-43 regulate distinct downstream cascades for neurite pruning.

7 Expanding the boundaries of memory: Sleep-dependent storage beyond the brain in *C. elegans*

Rashmi Chandra[1], Angel J Garcia[1], Evangeline Chein[2], Zhijian Ji[1], Bingying Wang[3], Junqiang Liu[3], Zihao Li[4], Betsy Chow[5], Fatema Mashel A Saifuddin[1], Julia M Miller[6], Kevin C Daigle[7], Ruben C Castro[7], Austin Edwards[8], Alexandra Lim[9], Vix Lou[9], Fisher Zhang[10], Wei Jiang[10], Winnie Yang[10], Yong Long[11], Michael Egan[11], Emily Soohoo[12], Miri K Van-Hoven[13], Torsten Wittmann[8], Christopher Fang-Yen[14], Dengke K Ma[10], Noelle D L'Etoile[151]University of California San Francisco, [2]University of Southern California, [3]Cardiovascular Research Institute, University of California San Francisco, [4]Ohio State University, [5]Cell and Tissue Biology, Student Education and Partnership, University of California San Francisco, [6]Neuroscience, University of California Los Angeles, [7]San Francisco State University, [8]Biological Imaging and Development Co Lab UCSF, University of California, [9]Cell and Tissue Biology, University of California, [10]Cardiovascular Research Institute, University of California, [11]University of California, [12]San Jose State University, [13]Department of Biological Sciences, San Jose State University, [14]Biomedical Engineering, Ohio State University, [15]Kavli Institute for Fundamental Neuroscience, University of California

Sleep and memory are essential components of human life, just as in *C. elegans*, which serves as a valuable model for investigating fundamental brain functions, including how sleep affects memory. Using a spaced olfactory training paradigm alongside detailed genetic and neural circuit analyses, I discovered that animals with healthy sleep patterns are more likely to remember to avoid an otherwise appealing odor (butanone) when olfactory training is paired with stressful stimuli such as exercise and starvation. Sleep-dependent memory relies on synaptic downscaling, and impairments in phagocytosis lead to sleeplessness and memory deficits without disrupting the learning process. Genetic, molecular, and optogenetic manipulations indicate that ALA neurons, CEPsh glia, and the hypodermis regulate sleep-dependent memory without impacting learning. This suggests that the memory trace of an odor may not be solely controlled by neurons or the brain; it can also be shaped or influenced by glia and non-neural cells through body-brain communication. Future experiments will examine how glial and non-neural cells contribute to memory consolidation via body-brain communication, focusing on the molecular pathways that mediate synaptic downscaling and sleep homeostasis. Additionally, I will explore how metabolic and environmental factors affect this process, offering insights into conserved mechanisms of sleep-dependent memory across species.

8 Examining the role of chemical neurotransmission on *C. elegans* post-embryonic nervous system maturation

Gabrielle S Prince[1], Ananya Theneti[1], Roger Pocock[2], HaoSheng Sun[31]Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, [2]Monash University, [3]University of Alabama at Birmingham

The number and composition of post-mitotic neurons remain largely unchanged from birth to adulthood, yet these neurons undergo extensive molecular and functional maturation changes during post-embryonic development, leading to distinct behavioral outputs. Disruptions in these processes can result in neurodevelopmental and psychiatric disorders. Previous research in vertebrate systems has highlighted the importance of external environmental stimuli on nervous system maturation, as deprived sensory stimulation during early post-embryonic/natal periods results in weakened synapses and aberrant synaptic pruning through neuronal activity/communication-dependent mechanisms. Additionally, neuronal activities during embryonic/fetal periods have been shown to be important in neural circuit maturation. Leveraging the powerful spatiotemporal genetics tools in *C. elegans*, we aim to comprehensively characterize the role of one major component of neuron-neuron communication, chemical neurotransmission, in nervous system maturation. Using the Auxin Inducible Degradation (AID) genetic system and an automated, high-resolution worm-tracking system, we systematically disrupted all classical modes of chemical neurotransmitter- and neuropeptide-based communication during either only embryonic or larval development and observed maturation impairments of locomotor behavior in adult worms. Concurrently, we are examining whether altered neuron-neuron communication intersects with cell-autonomous genetic timers, namely the heterochronic pathway, to regulate post-embryonic neuronal maturation. Future directions of the study include refining the critical/sensitive periods and specific circuits involved in locomotion maturation, as well as further dissecting how altered chemical neuron-neuron communication impairs neuronal maturation at the synaptic and molecular levels. Our study aims to provide a comprehensive characterization of the what, when, where, and how chemical neuron-neuron communication regulates behavioral maturation in adulthood. This research will advance our understanding of mechanisms controlling nervous system maturation and provide useful insights for neural disorder therapeutics.

9 Dissecting the Molecular, Functional, and Regulatory Properties of Novel "Heterochannel" Electrical Synapses

Atal Vats[1], Sudhanand Muraleedharan[1], Shivangi Verma[1], Nayantara Varma[2], Vatsala Thirumalai[1], Sandhya P Koushika[2], Abhishek Bhattacharya[11]National Centre for Biological Sciences, [2]Department of Biological Sciences, Tata Institute of Fundamental Research

Hard-wired neuronal communication relies on the precise functioning of both chemical and electrical synapses. However, despite their critical roles in circuit development and function, electrical synapses have been relatively understudied compared to their chemical counterparts. Electrical synapses are formed by clusters of gap junction channels, composed of transmembrane proteins called connexins (vertebrates) and innexins (invertebrates). Evidences from both vertebrates and invertebrates suggest that individual neurons can express multiple different innexins/connexins. However, the underlying significance of this diverse expression remains poorly understood. Hence, we asked "How does a neuron utilise its connexin/innexin repertoire to make meaningful connections with distinct synaptic partners?"

To achieve this, we dissected the molecular and functional identities of electrical synapses in the posterior touch-receptor neuron, PLM. Our data for the very first time revealed that individual electrical synapses can be "heterochannelled" in nature, i.e., they can be formed by clustering molecularly distinct gap junction channel, made up of different innexins. Further, we find that molecularly distinct channels within an electrical synapse:

Contribute differentially towards generating the mechanosensory behaviour of the animal.

Can be differentially regulated by actin cytoskeleton and by distinct regulatory machineries proposed to facilitate channel turnover.

Undergo reversible changes in their molecular composition in synapse-specific manner under adverse environmental conditions (dauers), resulting in novel mode of synaptic plasticity.

Our data also provides evidence for the presence of heterochannel electrical synapses beyond the mechanosensory circuit, suggesting a diverse and combinatorial function of innexins that can be investigated further. Moreover, we extended our study to Zebrafish Purkinje neurons, that also simultaneously expresses multiple connexins. Our data suggests the presence of heterochannel electrical synapses in these neurons as well, highlighting the conservation of this phenomenon across different organisms. Taken together, our work provides novel insights into the molecular and functional nature, regulation, and plasticity of electrical synapses. From a broader perspective, these findings also contribute to our current understanding of the genetic basis of phenotypic variability in electrical synapse related disorders.

10 Cell-extrinsic and intrinsic mechanisms of axon initial segment development

Kelsie Eichel, Emily VanLeeuwen, Lenissa Alcantara University of Colorado Boulder

Nervous system function requires exquisite spatial organization of neurons for proper signal transmission. Key to this signal transmission is a critical neuronal domain called the axon initial segment (AIS) that maintains polarity and generates action potentials. Disruption of the AIS structure is linked to a wide array of neurological disorders. The prevailing view over the last decades is that AIS development occurs through cell-intrinsic mechanisms within the neuron—a view largely stemming from neurons isolated from their native environment and grown in culture. We previously found that *C. elegans* neurons have hallmark features of an AIS (Eichel et al. Nature 2022), establishing a novel system to study the AIS in an intact living animal in which extracellular interactions are maintained. Here, we leverage this *in vivo* system to investigate AIS developmental mechanisms. We find that the AIS, as defined by an enrichment of an ortholog of ankyrin/UNC-44L, a key AIS protein, develops in a stereotyped region and similar tissue environment in distinct neurons. Because the AIS of different neurons forms in a specific region of extracellular space, it suggests that AIS-determining factors exist in that extracellular space. In support of this, we find that the conserved heparin sulfate proteoglycan perlecan/UNC-52, an extracellular matrix protein, overlaps with the location of the AIS in multiple neurons. Loss of function mutations in *perlecan/unc-52* disrupts AIS development and function, causing both a decrease in ankyrin/UNC-44L localization at the AIS and a loss of neuronal polarity. We then found that perlecan functions in a pathway parallel to intracellular regulators of AIS development. Individual loss of function mutations in *perlecan/unc-52* or *β-spectrin/unc-70*, which represent extracellular and intracellular components, respectively, both significantly decrease ankyrin/UNC-44L accumulation at the AIS. Double mutation of both components causes a complete loss of ankyrin/UNC-44L enrichment at the AIS. Therefore, we propose a new model of AIS development that requires the convergence of extracellular and intracellular mechanisms to build this essential AIS structure *in vivo*. Future work will identify the molecular underpinnings of these converging mechanisms, unify *in vivo* and *in vitro* approaches to study AIS development, and investigate the functional consequences of such a stereotyped patterning of the AIS across the nervous system.

11 Non-canonical mechanisms of Toll-like receptor function in the chemosensory nervous system

Brian Griffin[1], Elver Ho[2], Niels Ringstad[21]Cell Biology, NYU School of Medicine, [2]Cell Biology and Neuroscience Institute, NYU School of Medicine

Toll-like receptors (TLRs) are an evolutionarily ancient class of cell-surface molecules with well characterized roles in morphogen signaling and innate immunity. In these contexts, TLRs use a canonical signaling mechanism that involves recruitment of adapter proteins to the cytoplasmic domain of TLRs and subsequent activation of a kinase cascade that regulates transcriptional responses. Recently, we and others have found that TLRs are required for normal development and function of sensory circuits. Unlike morphogen signaling and innate immunity, sensory circuit development does not require canonical TLR signaling mechanisms. The non-canonical mechanisms by which TLRs regulate the development of sensory circuits are unknown. Here, we show evidence that the sole TLR encoded by the *C. elegans* genome, TOL-1, functions in neurodevelopment and we propose a plan to determine non-canonical mechanisms of TLR signaling in sensory neurons. TOL-1/TLR is highly enriched in the developing neuropil of the embryonic nervous system, preceding synaptogenesis and persisting through late-stage embryonic development. Using a genetically engineered conditional allele of *tol-1* via the ZIF-1/ZF1 degron system, I found that pan-neuronal depletion of TOL-1/TLR disrupts neuropil organization and causes marked defects in CO2 chemotaxis behavior. Further, TOL-1/TLR functions cell autonomously in the CO2 chemosensory neurons, BAGs, to regulate this behavior. Lastly, TOL-1/TLR does not require its intracellular domain to function in sensory neurons, indicating that its function is independent of canonical TLR signaling mechanisms. We hope to leverage the phenotypes exhibited by animals lacking neuronal TOL-1/TLR to identify factors that mediate non-canonical TLR signaling and the role that TOL-1/TLR plays in chemosensory neurodevelopment.

12 Shaping axons with intestinal lipids

Wenyue Wang, Roger Pocock Monash University

Lipids are integral to cell membrane structure and function, serving key roles in diverse biological signalling pathways. In the nervous system, disrupted lipid homeostasis causes axon fragility; however, how lipid homeostasis impacts axon development is not fully understood.

Here, we found that sphingolipids, a group of bioactive lipids derived from the intestine, control axon development and plasticity. Animals lacking the acid ceramidase ASAH-1, a key sphingolipid metabolic enzyme, have misguided axons just after hatching. Remarkably, however, these axon defects are completely resolved within 16 hours of post-embryonic development – revealing a form axon plasticity. Loss of ASAH-1 would likely cause accumulation of ceramides, and potentially other lipids such as sphingomyelin. We thus examined axon development in genetic mutants of sphingomyelinases (ASM-1, ASM-2, ASM-3), which convert sphingomyelin into ceramide. Loss of ASM-2 phenocopies the axon developmental defects of animals lacking ASAH-1 and these defects are also resolved within 16 hours of post-embryonic development. This suggests that sphingomyelin accumulation is responsible for this axon developmental defect. We validated this hypothesis by suppressing the *asm-2* mutant-induced axon defects through loss of the sphingomyelin synthases SMS-1 or SMS-3, which would reduce sphingomyelin production. Using CRISPR-Cas9, we generated endogenous fluorescent reporters for ASAH-1 and ASM-2, both of which are expressed in the intestine. We found that the axon defects caused by *asah-1* or *asm-2* loss are rescued when their expression is restored in the intestine, suggesting a non-cell autonomous role of sphingolipids in regulating axon development. In subsequent mechanistic analysis, we have revealed specific axon guidance and extracellular matrix regulators that suppress or enhance axon defects caused by disrupted sphingolipid homeostasis. Taken together, our results show that intestinal sphingolipids control early axon development cell-non-autonomously and reveal a model for understanding processes that regulate developmental neuroplasticity.

13 Mechanical Anchoring of SYG-1/Neph1 and SAX-3/Robo Directs Neurite Branching in *C. elegans*

Yun-Hsien Lin, Chun-Hao Chen National Taiwan University

Adhesion molecules play a crucial role in guiding neurite outgrowth and establishing precise neural connections. Typically, these molecules transmit intracellular signals through their cytosolic domains to regulate cytoskeletal dynamics essential for neurite extension. In this study, we demonstrate that mechanical interactions between adhesion molecules SYG-1/Neph1 and SAX-3/Robo are sufficient to direct neurite branching in the nematode *Caenorhabditis elegans* (*C. elegans*). PVPs are cholinergic interneurons that extend sexually dimorphic dorsal branches, attaching to vulE and vulF in hermaphrodites. Through a candidate screen, we find that mutations in *syg-1* result in reversed PVP branching, with projections misdirected toward the ventral nerve cord. Time-lapse imaging of *syg-1* mutants reveal a gradual reorientation of PVP branches coinciding with vulva development. SYG-1 functions autonomously to guide PVP branching, but unexpectedly, its role in branching depends solely on its immunoglobulin and transmembrane domains, while its cytosolic tail, containing an F-actin recruiting sequence, plays only a minimal role. This suggests that SYG-1 primarily anchors its ectodomain on PVP neurons to facilitate neurite branching. Moreover, we find that SYG-1 does not interact with its canonical receptor Nephrin/SYG-2 but instead engages Robo/SAX-3 acting in vulval epithelial cells in a common genetic pathway. Remarkably, swapping the expression of SYG-1 and SAX-3 between PVP neurons and the vulva in *syg-1* mutants rescues PVP branching defects, supporting an anchoring model in which mechanical interaction between SYG-1 and SAX-3, rather than intracellular signaling, is sufficient to direct neurite projection. This work provides new insights into adhesion-based signaling mechanisms that shape neurodevelopment.

14 Towards identifying the critical period for the embryonic origins of individualized behavior

Christopher Brittin, Zhirong Bao Memorial Sloan Kettering Cancer Center

Studies across multiple model systems (worms, fish and mice) have demonstrated that individualized patterns of behavior are measurable at birth within an isogenic population. These observations suggest that individualized behavior emerges within the embryo. To better characterize the critical period and corresponding developmental events within the embryo that bias individualized larval behavior, we have established an adaptive bright field imaging platform for monitoring perinatal behavior in *C. elegans*. Sampling at 0.3 Hz, our platform can image multiple embryos at low magnification (10x) and -- once hatching is detected -- switch to very low magnification (2.5x) for continued tracking of individual larval worms. Prior work has shown that early embryonic motility behavior ("twitch") starts at ~430 min post first cleavage (mpfc) and is myogenic. Between twitch and hatching, we are able to describe embryonic motility with a two-component mathematical model that captures the contribution of myogenic and neurogenic activity. Our model predicts two key critical points: T1 and T2 at ~530 and ~700 mpfc, respectively. Using a CND-1 membrane marker, we find that dorsal cord innervation by A and D-class motoneurons occurs between T1 and T2. The RAB-3 synaptic marker shows synaptic puncta transiently forming in the nerve cord before and after T2. Furthermore, both synaptic (*unc*-13) and neuropeptidergic (*unc*-31) mutants exhibit delays in T2 but not T1. Finally, we traced calcium activity in dorsal cord motoneurons before and after T2. Prior to T2, we find spontaneous "all-fire" bouts where most A, B and D class dorsal cord neurons are simultaneously active. Following T2 and consistent with larval motor activity, we do not observe these "all-fire" bouts but instead observe that A and B class neurons correlate with backward and forward motion within the embryo, respectively. Collectively, these results suggest that the critical window between T1 and T2 encompasses the transition from myogenic to neurogenic activity. Ongoing work is assessing whether developmental events during this critical window are predictive of larval behavior.

15 Glial Neuropeptide Signaling and Non-Canonical Release Mechanisms

Tepary Cooley[1], Kylie Boor[2], Tatum Joyner[2], Maria Purice[21]Biochemistry and Biophysics, Oregon State University, [2]Oregon State University

Glia play fundamental roles in brain function across species, yet the molecular mechanisms by which they modulate neuronal activity and behavior remain poorly understood. To address this question, we performed snRNA-seq on *C. elegans* glia spanning the entire nervous system of young adult hermaphrodites and males, revealing diverse transcriptional signatures (see the complete atlas at wormglia.org). Among these, we identified the expression of neuropeptides, a class of signaling molecules whose processing and function in glia remain largely unexplored.

Our data reveal heterogeneous expression of neuropeptides among glial subtypes, raising the possibility that neuropeptide signaling contributes to glial diversity. We also identified two proprotein convertases, *kpc-1* and *bli-4*, in our dataset and confirmed *bli-4* expression in a subset of glia in vivo, suggesting a role in neuropeptide processing. Additionally, both in silico and in vivo analyses show that UNC-31/CAPS, a calcium-dependent dense-core vesicle secretion factor essential for neuropeptide release in neurons, is not present in glia. This finding suggests that glia rely on a fundamentally distinct, non-canonical mechanism for neuropeptide secretion.

Building on candidates identified in our snRNA-seq dataset, we are investigating this alternative neuromodulatory release pathway in glia. In parallel, we are exploring the role of glial neuropeptides in neuronal function, aiming to uncover how glia contribute to neural circuit modulation. Understanding these mechanisms may not only advance our knowledge of glial function in *C. elegans* but also offer insights into conserved neuropeptide signaling pathways.

16 Innexin gap junctions carry a cytosolic cap structure consisting of a ring of UNC-1 Proteins

Nils Rosenkranz[1], Konstantin Wieland[1], Alexandra Birtasu[1], Sina Manger[1], Abhishek Bhattacharya[2], Achilleas Frangakis[1], Alexander Gottschalk[11]Buchmann Institute, Goethe University, [2]National Centre for Biological Sciences Tata Institute of Fundamental Research

Innexins form gap junctions that directly connect the cytosols of neighboring cells. They are essential for orchestrating and/or synchronizing electrical activity in neurons and muscle ensembles, and can transfer metabolites and second messengers between cells. *C. elegans* has 25 innexin genes, expressed in diverse tissues. Electron microscopy data of negative-stained tissue has demonstrated hexagonal arrangements of putative innexin channels in intestinal membranes, forming large patches. However, the molecular structure of gap junctions *in situ*, by cryo-electron tomography of unstained samples, is unknown. Here, we have identified and imaged innexin gap junctions in cultured *C. elegans* cells. We find gap junctions with typical 3-5 nm intermembrane distances, which contain protein density spanning both adjacent membranes, with a central channel. Sub-tomogram averaging of > 1500 channels resulted in a 3D surface map with the typical, hourglass shape of gap junction channels. These appear in wide and narrow configurations, possibly representing open and closed states. The most abundant gap junctions consist of UNC-7 and UNC-9 innexins. For UNC-9, a functional and physical interaction with the stomatin protein UNC-1 was reported (PMIDs: 17658257, 28143932). Mutations in *unc-1* exert dominant negative effects on UNC-9 function. Stomatin proteins were recently shown to assume multimeric, ring-like assemblies that attach to membranes (by lipid anchors, or single TM helices), forming enclosed compartments. Interestingly, we find a subset of gap-junction channels that carry a cytosolic protein complex, resembling the Alphafold3 structural model of an UNC-1 hexadecamer. We suggest that UNC-1 forms a cap-like structure covering the cytosolic opening of the gap junction channel. These caps occur on either side of the channel, or on both. We analyzed gap junctions from animals that express N-terminally GFP-tagged UNC-1. In tomograms, we sometimes observe extra-density at the cytosolic side of the cap/gap junction complex, making it likely that the cap consists of UNC-1. Furthermore, we expressed UNC-1 and UNC-9 in HEK cells and find capped gap junction channels at cell-cell interfaces. While the functional meaning of this molecular interaction is as yet unknown, our work lays out a path for further analysis of the essential role of the UNC-1 stomatin – innexin interactions.

17 An activity-dependent transcriptional program coupled with coordinated mRNA export drives synaptogenesis during development

Callista Yee[1,2], Yutong Xiao[3], David Matus[3,4], Kang Shen[1,2,5]1Stanford University, [2]Howard Hughes Medical Institute, [3]Stony Brook University, [4]University of California, Berkeley, [5]Wu Tsai Neurosciences Institute

Synapse formation requires rapid and coordinated production of hundreds of synaptic proteins. This process not only involves transcriptional activation, mRNA export and translation, but also requires control by neuronal activity. Our group has previously identified the conserved THO nuclear export complex (THOC) as an important regulator of presynapse development in *C. elegans* and dopaminergic neuron synapse maintenance in mice (Maeder *et al.*, 2019). Our study was the first to demonstrate that nuclear export is a critical rate-limiting step for synaptogenesis and neuronal differentiation. One critical question that was not addressed in this study is how the THO complex selects its mRNA targets.

Using *C. elegans* dopaminergic neurons, we report that synaptic gene expression is controlled by neuronal activity and by two transcription factors (TFs), the AP-1 protein FOS-1/Fos and the zinc finger TF EGL-43/MECOM. Through cell-specific TF-profiling techniques, we find that both EGL-43 and FOS-1 bind directly to upstream regulatory elements of presynaptic genes to activate transcription. Depletion of either TF or mutation of their binding sites on presynaptic loci severely affects presynaptic gene expression *in vivo*. Using endogenous GFP-tagged proteins, we demonstrate that EGL-43 and FOS-1 regulate each other's expression. With a single nucleotide change in the FOS-1 binding site upstream of *egl-43*, we show that enhancing FOS-1 binding is sufficient to dramatically increase expression of EGL-43 and synaptic proteins. Consistent with mammalian interaction studies, we report that EGL-43 interacts with subunits of THOC. We demonstrate the ability to confer binding of mRNAs to THOC *in vivo* through the insertion of EGL-43 binding sites upstream of housekeeping genes. With this mechanism, EGL-43 provides mRNA target specificity to THOC to facilitate export of presynaptic mRNAs. Together, we describe the first evidence of a transcription factor and RNA export machinery directly controlling the expression of functional components of the synapse during development.

18 Dissecting an intestinal scaffold for commensal bacterial adherence in the lumen

Anupama Singh[1], Dalaena Rivera[1], Ophélie Nicolle[2], Grégoire Michaux[2], Robert Luallen[11]Department of Biology, San Diego State University, [2]Univ Rennes, CNRS, IGDR (Institut de Génétique et Dévelopement de Rennes)

Bacterial adherence in the gut is a potential host selection mechanism to facilitate microbial retention and colonization in the gut. However, the host genes and pathways involved in bacterial adherence and colonization are less understood. By sampling *C. elegans* from the wild, we have isolated a novel commensal bacteria, *Lelliottia jeotgali*, that colonized lab grown wild-type *C. elegans* by adhering to their intestinal lumen. We are using this natural *C. elegans* microbiome bacteria to decipher *C. elegans* genes and pathways that regulate bacterial adherence/colonization.

To identify *C. elegans* genes required for bacterial adherence in the gut, we conducted a forward genetic screen to select mutants that lost colonization by *L. jeotgali*. We identified multiple allelic mutations in at least five different *C. elegans* genes, termed knockout of *Lelliottia* adherence *(kola)* mutants. These genes encode a predicted membrane bound mucin (KOLA-1) and multiple secreted proteins with VWFA domains (KOLA2, 4-5). Interestingly, the secreted KOLA2-5 proteins were predicted to interact with each other by STRING analysis, and each were found to be in the intestinal proteome. Based on this data, we hypothesize that KOLA2-5 are utilized as a scaffolded receptor for bacterial adherence in the gut lumen.

To understand microbiome-adherence driven changes in the *C. elegans* gut we implemented TEM. We found that wild-type animals colonized with *L. jeotgali* had a more circular and distended intestinal lumen compared to uncolonized animals fed OP50, but had normal microvilli. We could visualize *L. jeotgali* attaching to the glycocalyx above the microvilli and dividing in the lumen. By contrast, two of our *kola* mutants had elliptical lumens that looked similar to OP50-fed controls. Strikingly, wild-type animals were observed to contain a thin, electron-dense structure above the glycocalyx that was absent in two *kola* mutants. Additionally, we tested two other adherent microbiome bacteria in different taxa (*Ca.* Lumenectis limosiae, and *Ca.* Enterosymbion pterelaium) that still bound to our *kola* mutants, suggesting that there is specificity to microbial binding in the gut lumen. We plan to further dissect the molecular composition of this intestinal scaffold and understand the role of *kola* genes in microbiome adherence. In addition, we are investigating the impact of microbiome bacterial adherence on *C. elegans* physiology and health.

19 Pathogen apathy: how depletion of serotonin from SKN-1 activation modifies behavior

Tripti Nair, Brandy A Weathers, Nicole L Stuhr, James D Nhan Leonard Davis School of Gerontology, University of Southern California

When an organism encounters a pathogen, the host innate immune system activates to defend against pathogen colonization and toxic xenobiotics produced. *C. elegans* employs multiple defense systems to ensure survival when exposed to *Pseudomonas aeruginosa*, including activation of the cytoprotective transcription factor SKN-1/NRF2. Although wildtype *C. elegans* quickly learn to avoid pathogens, here we describe a peculiar apathy-like behavior towards PA14 in animals with constitutive activation of SKN-1, whereby animals choose not to leave and continue to feed on the pathogen even when a non-pathogenic and healthspan-promoting food option is available. Although lacking the urgency to escape the infectious environment, animals with constitutive SKN-1 activity are not oblivious to the presence of the pathogen and display the typical pathogen-induced intestinal distension and eventual demise. SKN-1 activation, specifically in neurons and intestinal tissues, orchestrates a unique transcriptional program, which leads to defects in serotonin signaling that is required from both neurons and non-neuronal tissues. Serotonin depletion from SKN-1 activation limits pathogen defense capacity, drives the pathogen-associated apathy behaviors and induces a synthetic sensitivity to selective serotonin reuptake inhibitors. Finally, we define new dimensions of serotonin receptor signaling in the context of constitutive SKN-1 activity and host-pathogen responses. Taken together, our work reveals an intriguing new SKN-1-dependent homeostatic circuit that responds to environmental pathogens and is mediated by serotonin to promote survival.

20 *C. elegans* innate immune OFF/ON switch proteins PALS-22 and -25 localize to mitochondria

Max W. Strul[1], Spencer S. Gang[2], Emily R. Troemel[11]Biological Sciences, University of California San Diego, [2]Colorado College

Mitochondria, well known as "the powerhouse of the cell", are perhaps lesser known as an important signaling hub for the innate immune system. In mammals, pattern recognition receptors (PRR) pathways utilize mitochondria as a platform, such as RIG-I/MAVS. Although *C. elegans* lack canonical MAVS/NFkB signaling, here, in unpublished data, we report that PALS-22 and PALS-25, which regulate the intracellular pathogen response (IPR), localize to mitochondria, despite lacking canonical mitochondrial targeting sequences. Previous work in the lab characterized PALS-22 and PALS-25 function as an OFF/ON switch for the IPR, respectively. Specifically, loss of the IPR inhibitor, PALS-22, promotes immunity against intracellular pathogens which is reversed by loss of the IPR activator, PALS-25 (Reddy 2019, PLOS Pathogens). Here, in unpublished data, we show that PALS-22 requires PALS-25 to localize to mitochondria, while PALS-25 still localizes to mitochondria in the absence of PALS-22, although it forms discrete puncta of unknown significance. Based on structural predictions by AlphaFold, we cut PALS-25 into two domain halves. We found that the C-terminal half of PALS-25 is necessary and sufficient for mitochondrial localization. Further, we found that the N-terminal 40 amino acids of PALS-25 appear to be required for IPR signaling. We also found that *pals-22* mutant animals have altered mitochondrial morphology. Kinetic studies revealed that mitochondrial morphology changes induced by loss of PALS-22 occur after IPR gene induction, and thus morphology changes are likely downstream of the IPR. To further test this model, we induced the IPR through knockdown of another previously published IPR negative regulator, *pals-17*, which localizes to the plasma membrane, which here we found that this also induced mitochondrial morphology changes. Our results show two more IPR regulators localize to membranes and that downstream of the IPR we see mitochondrial morphology changes. Future studies aim to characterize how these proteins induce the immune response.

21 *Stenotrophomonas indicatrix* Induces Intracellular Pathogen Defense in *Caenorhabditis elegans*

Jordan West[1], Samuel Schwartz[2], Samuel Li[2], Peri Roper[2], Vladimir Lazetic[21]Biological Sciences, The George Washington University, [2]The George Washington University

The microbiome plays a crucial role in shaping host immunity, but the mechanisms by which specific bacteria modulate innate immune pathways remain poorly understood. *Caenorhabditis elegans*, a bacterivore with a well-defined microbiome, provides an ideal model for dissecting the role of the microbiome in modulating host-pathogen defense mechanisms. One such defense mechanism in *C. elegans* is the Intracellular Pathogen Response (IPR), a transcriptional immune response activated upon infection by obligate intracellular pathogens such as the fugal parasite *Nematocida parisii* and Orsay virus. The IPR coordinates the upregulation of multiple genes, including *pals-5*, whose promoter region was used to create a fluorescent transcriptional reporter for IPR activation. We screened 12 representative microbiome species for their ability to induce *pals-5p::GFP expression*, and we found that *Stenotrophomonas indicatrix* (JUb19) promotes IPR reporter expression in multiple tissues, including the intestine, epidermis, neurons, and somatic gonad. Because all previously described IPR triggers depend on the bZIP transcription factor ZIP-1 for *pals-5*p::GFP expression, we tested whether JUb19 also requires ZIP-1. We found that ZIP-1 is only needed during the early phase of IPR reporter induction by JUb19, suggesting that additional transcription factors regulate *pals-5p::GFP* expression. When investigating how JUb19 induces IPR *reporter* expression, we found that JUb19 colonizes the intestinal lumen in adult animals without invading host cells, making it the first microbe known to activate *pals-5p::GFP* expressionextracellularly. Interestingly, while heat-killed JUb19 fails to induce reporter expression, mechanically disrupted and chemically inactivated JUb19 retains this ability. This suggests that IPR activation is triggered by a molecular component of JUb19 that is sensitive to heat. Our preliminary results indicate that this component may be bacterial RNA specific to JUb19. Our transcriptomic analyses demonstrate that JUb19 induces the expression of a small subset of IPR genes, as well as genes with lysosome-associated functions. Importantly, these transcriptional changes are sufficient to protect the host against both viral and fungal pathogens based on our infection assay results. However, JUb19 also causes fitness disadvantages, including developmental delay, shorter lifespan, and reduced brood size. Together, our study identifies JUb19 as a component of the *C. elegans* natural microbiome that induces a novel transcriptional response, promoting innate immunity against obligate intracellular pathogens.

22 Redundant, non-cell-autonomous regulation of the oomycete recognition response

Manish Grover, Kenneth Liu, Florence Drury, Michalis Barkoulas Imperial College London

How *C. elegans* senses various pathogens to mount pathogen-specific immune responses is not well understood. We investigate this problem in the context of the nematode's natural infection by oomycetes, which are eukaryotic pathogens that morphologically resemble fungi although they are phylogenetically distinct. Much of our understanding of the oomycete biology comes from studies on *Phytophthora infestans*, the pathogen responsible for potato late blight and the Great Irish Famine. However, oomycetes also pose a significant threat to animal health, particularly in tropical regions where *Pythium insidiosum* infects mammals, including humans. Previous research revealed that *C. elegans* recognizes oomycete pathogens and initiates a protective transcriptional program named as the oomycete recognition response (ORR), which is characterized by the induction of multiple chitinase-like (*chil*) genes in the epidermis. Using forward genetic screens, we demonstrate that pairs of transmembrane C-type lectin receptors essential for pathogen recognition. For instance, a CLEC-27/CLEC-35 pair is required for animals to detect *Myzocytiopsis humicola*, while CLEC-26/CLEC-36 recognizes *Haptoglossa zoospora*. Pathogen recognition occurs in sensory neurons and the intestine in a redundant manner and is able to trigger an epidermal signalling cascade mediated by the kinase-pseudokinase pair OLD-1/FLOR-1. The CLEC receptor pairs, and the OLD-1/FLOR-1 module are regulated by the homeodomain proteins CEH-37/OTX2 and VAB-3/PAX-6 respectively, which play critical roles in mammalian visual system development. In conclusion, our work uncovers how a combination of conserved and species-specific factors orchestrates defence responses against oomycete pathogens. This research offers broader insights into pathogen recognition strategies in *C. elegans* and oomycete infections of animal hosts.

23 Yersinia biofilm-dependent killing reveals functional ncRNA polymorphism and social transfer of glycosylated surface material

Jonathan Hodgkin[1], Samuel Noah Harris[1], Tomos Rodrigues[1], Nabeel S. Ganem[21]Biochemistry, Univ Oxford, [2]Univ Oxford

Adult *C. elegans* exposed to lawns of the widespread and promiscuous bacterial pathogen, *Yersinia pseudotuberculosis* (YP), may rapidly accumulate surface biofilms and then die within a day as a result of cuticular destruction. Bristol (N2) worms are highly sensitive to YP but most (31/40) wild isolates, such as Hawaiian (CB4856), are resistant and grow well on YP lawns. Sensitive strains have been found in England, France, Spain, Madagascar, Australia and California. CB4856/N2 introgression lines and SNP mapping identified a major resistance locus, *yakr-1* (Yersinia Adult Killing Resistant) located in a small region on LG1. This 40 kb region lacks obvious coding polymorphisms but harbors a predicted non-coding circular RNA gene that contains a SNP strongly correlated with resistance in multiple wild isolates. Mutations in several genes affecting pathogen susceptibility and surface glycosylation (3 *bah*, 6 *bus*, 2 *srf* genes) confer complete resistance to biofilm accumulation and YP killing. We hypothesize that *yakr-1* may act by down-regulating one or more of these genes.

Resistant Hawaiian worms that have cohabited with worms from sensitive strains gradually become sensitive, as a result of contact-dependent transfer of surface material. Social clumping increases the efficiency of this transfer. Similarly, resistant *bah*, *bus* or *srf* worms become sensitive to YP after a few hours of contact with sensitive worms or with cell-free debris from lysed sensitive worms. The biofilm-inducing material ('BIM') is insoluble and resistant to alkali, heat treatment and proteolysis. BIM may be a complex surface glycan, which can be transferred between worms by direct contact.

24 CHN-1 knock-out impairs TBP proteasomal degradation leading to functional alteration of mechanosensory neurons.

Giuliana Madonna[1,2], Pamela Santonicola[3], Elia Di Schiavi[3], Franco Taroni[1], Stefania Magri[11]Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, [2]Department of Medicine and Surgery, University of Milano-Bicocca, [3]Institute of Biosciences and BioResources (IBBR)-National Research Council of Italy (CNR)

The human transcription factor TATA-box binding protein (*TBP*) gene is characterized by a polyQ-encoding CAG/CAA repeat whose expansion causes the autosomal dominant spinocerebellar ataxia type 17 (SCA17). Interestingly, while ≥47 polyQ repeats are fully-penetrant pathogenic alleles, 40-46 repeats show reduced penetrance and are thus defined intermediate alleles. We have recently described that affected patients with intermediate alleles carried a concurrent heterozygous pathogenic variant in *STUB1* gene demonstrating that SCA17 is monogenic for TBP with ≥47 polyQ and digenic *TBP/STUB1* for intermediate alleles (SCA17digenic). *STUB1* encodes the C-terminus Hsp70-interacting protein (CHIP), an E3-ubiquitin ligase with co-chaperone activity. To unravel the nature of this interaction, we have generated *Caenorhabditis elegans* SCA17 models by pan-neuronal overexpression of the cDNA of human *TBP* alleles with 38 (wild-type, TBPWT), 43 (intermediate, TBPQ43), and 54 repeats (fully-penetrant, TBPQ54). We observed that only the fully-penetrant pathogenic polyQ expansion caused functional alteration of mechanosensory neurons. Instead, knock-out of CHN-1 (*chn-1(by155)*), orthologue of human CHIP, compromised wild-type backward locomotion, consistent with the lower number of visible GABAergic motor neurons observed. Interestingly, the SCA17digenic model *TBPQ43;chn-1(by155)* showed an age-dependent defect in gentle touch response, similarly to TBPQ54 animals. Moreover, treatment of TBPQ43 animals with the proteasomal inhibitor MG132, but not with the lysosomal inhibitor chloroquine, impaired gentle touch response resulting in a defective phenotype similar to SCA17digenic model. These results support the hypothesis that CHIP/CHN-1 mediates TBP/TBP-1 proteasomal degradation. We have also confirmed, in single-copy integrated model of SCA17digenic, the functional alteration of mechanosensory neurons present in the overexpressed model, thus indicating that the phenotype observed is determined by TBP mutation rather than its overexpression. In conclusion, we have generated the first SCA17 and SCA17digenic *C. elegans* models that recapitulate disease pathogenicity. In particular, we observed that, although intermediate polyQ expansion in TBP are not sufficient to cause an altered phenotype, CHN-1 absence impairs TBPQ43 proteasomal degradation triggering disease onset. Our model will be used to identify other players involved and new treatments able to rescue the altered phenotype.

25 Activation of *C. elegans* fatty acid desaturation pathway by dietary sugar is dependent on *E. coli* Pyruvate Dehydrogenase Complex

Marco E Mechan Llontop[1], Kelsie M Nauta[2], Darrick R Gates[2], Xiao Wang[2], Nick O Burton[21]Van Andel Institute, [2]Department of Metabolism and Nutritional Programming, Van Andel Institute

Host metabolism is intricately regulated by dietary composition and the subsequent processing of nutrients by the host's microbiome. While high-sugar diets are known to modulate insulin signaling and lipid metabolism in *Caenorhabditis elegans,* the role of commensal bacteria in regulating these effects remains poorly understood. Here, we demonstrate that the metabolic state of *Escherichia coli* OP50 determines the physiological response of *C. elegans daf-2* (insulin receptor) mutants to a high-sugar diet. Specifically, previous studies demonstrated that high sugar diets can overcome many of the developmental and aging defects observed in *daf-2* mutant animals. These effects were presumed to be due to dietary sugar increasing animal insulin signaling which could overcome partial loss-of-function of the DAF-2 insulin receptor. Surprisingly, we find that the effects of dietary sugar on animals is dependent on the activity of the Pyruvate Dehydrogenase Complex (PDC) in microbiome bacteria. This includes finding that disruption of the Pyruvate Dehydrogenase Complex subunit *ΔaceE* can suppresses the defects observed in *daf-2* mutants even in the absence of high sugar and that the presence of high sugar can no longer suppress *daf-2* to mutants in the presence of *ΔaceE* mutant *E. coli.* We further used transcriptomic analysis to reveal that bacterial PDC disruption prevents a high sugar diet from triggering the induction of rate limiting enzymes involved in fatty acid desaturation including *fat-5*, *fat-7*, *far-3* and *acdh-2*. Collectively, these findings suggest that bacterial PDC activity plays a pivotal role in modulating host lipid metabolism in response to dietary sugar availability. Our findings reveal a critical microbial influence on host metabolic regulation, demonstrating that bacterial pyruvate dehydrogenase activity directly shapes the effects of dietary sugars on host physiology. These insights deepen our understanding of host-microbiome interactions and offer potential avenues for microbiome-based metabolic interventions.

26 *gda-1* and *gda-2* encode paralogous guanine deaminases that act together to maintain purine homeostasis in *C. elegans*

Sushila Bhattacharya[1], Jennifer Snoozy[1], Lisa Fischer[2], Marco Herde[2], Grace Hagedorn[1], Claus-Peter Witte[2], Warnhoff Kurt[1,31]Sanford Research, [2]Leibniz University Hannover, [3]Department of Pediatrics,Sanford School of Medicine, Sanford School of Medicine, University of South Dakota

Purines are fundamental building blocks for DNA and RNA, and failures in purine homeostasis lead to rare and common human diseases such as Lesch-Nyhan syndrome, xanthinuria, gout, and cancer. Thus, understanding the metabolic networks that govern purine synthesis, degradation, and salvage is an important goal. We have developed a *C. elegans* model for xanthine dehydrogenase (*xdh-1*) deficiency/xanthinuria. Interestingly, *xdh-1* mutant *C. elegans* develop rare xanthine stones, mirroring pathology observed in humans. To identify genetic regulators of purine homeostasis, we performed a screen for mutations that enhance the formation of xanthine stones in *xdh-1* mutant animals. We identified multiple loss-of-function mutations in a putative guanine deaminase which we have named *gda-1*. Here, we provide genetic and biochemical evidence that *gda-1* indeed encodes an intestinal enzyme with guanine deaminase activity and functions as a critical component of *C. elegans* purine catabolism. Interestingly, our bioinformatics analyses suggest that *gda-1* found in the nematode lineage was acquired by horizontal gene transfer from prokaryotes. Supporting this model, *gda-1* loss of function can be rescued by transgenic expression of a homologous prokaryotic guanine deaminase derived from *Bacillus subtilis.* Interestingly, the *C. elegans* genome encodes a *gda-1* paralog, *gda-2*. *gda-2* functionally complements *gda-1* loss of function but is expressed in distinct tissues, suggesting that the specialization of expression pattern is the driver of evolutionary maintenance of these paralogs. Furthermore, despite playing redundant roles in promoting guanine homeostasis, *gda-1* and *gda-2* play complex and non-overlapping roles in controlling xanthine accumulation. Together, our studies characterize the function of *gda-1* and *gda-2* in *C. elegans* metabolism and suggest an evolutionary model whereby a bacterial gene was co-opted by nematodes to influence purine catabolism, a fundamental metabolic pathway.

27 Constitutive expression of the mitochondrial unfolded protein response protects against germ cell ferroptosis in *C. elegans*

Jimena Ruiz[1], Jennifer Watts[21]School of Molecular Biosciences, Washington State University, [2]Washington State University

Ferroptosis is a unique form of iron-dependent cell death; its defining feature being the toxic buildup of lipid peroxides. Within the plasma membrane, various polyunsaturated fatty acids (PUFAs)-containing phospholipids can be peroxidized. Additionally, under ferroptosis-inducing conditions, lipid peroxides accumulate within organelles prior to membrane rupture. PUFAs have a greater susceptibility to lipid peroxidation than other fatty acids with just one or no double bonds. The Watts lab discovered that the w-6 PUFA, dihomo-g-linolenic acid (DGLA) causes germ cell death and sterility via ferroptosis in *Caenorhabditis elegans* (*C. elegans)*. Since the *C. elegans* germline is highly metabolically active and contains a vast number of mitochondria, which are a major source of reactive oxygen species (ROS), it makes a good model for investigating mitochondrial involvement in ferroptosis. We predict that mitochondria influence DGLA-induced ferroptosis in *C. elegans*. To investigate this, we obtained mutant strains with reduced mitochondrial electron transport chain function. Surprisingly, both complex I and complex II mutants were protective against DGLA-induced sterility. We tested whether this is due to the mitochondrial transcription factor ATFS-1 (ATF4 in mammals), which is responsible for maintaining mitochondrial homeostasis during times of stress by initiating the mitochondrial unfolded protein response. We found that constitutively active ATFS-1 mutants were highly resistant against DGLA-induced ferroptosis. To determine the mechanism of resistance, we are performing bulk RNA sequencing and confocal microscopy to visualize ROS and lipid peroxidation. Together, these studies aim to provide us with a better insight into the influences of mitochondrial stress responses on DGLA-induced ferroptosis to shed light on potential therapeutics for the several diseases associated with ferroptosis.

28 *mot-1* encodes a putative molybdate transporter required for embryonic viability in *C. elegans*

Robin R Fettig, Sushila Bhattacharya, Jennifer Snoozy, Kurt Warnhoff Pediatrics and Rare Diseases Group, Sanford Research

Molybdenum is an essential trace metal that is required for the synthesis of the molybdenum cofactor (Moco). Moco is required for animal life and is synthesized through an ancient and conserved biosynthetic pathway. In the final step of Moco synthesis, molybdenum insertase uses bioavailable molybdate to insert molybdenum into the MPT-AMP precursor, forming mature Moco. Despite well-characterized mechanisms of molybdate transport in prokaryotes, fungi, and plants, how animal cells acquire molybdenum for Moco synthesis remains mysterious. By evaluating distant homologs of plant molybdate transporters encoded by the *C. elegans* genome, we uncovered a transmembrane transporter that we have named molybdate transporter 1 (*mot-1*). *mot-1* is required for embryonic viability when animals are deprived of dietary Moco, suggesting a role for MOT-1 in promoting endogenous Moco synthesis. *mot-1* appears to act maternally; homozygous *mot-1* homozygous mutant embryos are viable when derived from heterozygous mothers. Furthermore, the embryonic lethality caused by *mot-1* loss of function is bypassed by supplemental molybdate, suggesting a defect in molybdate transport. Furthermore, the embryonic lethality displayed by *mot-1* mutant animals is suppressed by inactivating mutations in *cth-2* or *cdo-1,* established suppressors of Moco-deficient lethality. Biochemically, *mot-1* mutant embryos display reduced Moco content, as detected by reduced activity of the Moco-requiring sulfite oxidase enzyme. Together, these genetic and biochemical data suggest the model that MOT-1 is a *C. elegans* molybdate transport protein that is required in maternal tissue for promoting embryonic Moco homeostasis and viability. This is the first description of an animal molybdate transporter and lays the foundation for understanding molybdate transport in higher eukaryotes such as humans.

29 Tissue-specific responses to low PC allow an ARF GTPase “swap” as membrane properties change

Arjamand Mushtaq[1], Amy K Walker[21]PMM, UMASS Chan, [2]Program in Molecular Medicine, UMASS Medical School

Methylation is a central regulatory mechanism linking methionine and folate metabolism to gene expression through histone methylation and influencing membrane properties via phosphatidylcholine (PC) production. In *C. elegans*, the methyl donor S-adenosylmethionine (SAM) is synthesized by one of four SAM synthases: *sams-1*, *sams-3*, *sams-4*, and *sams-5*. One physiological pathway particularly sensitive to SAM levels is the cellular stress response. Each external stressor elicits a specific gene expression program, and we have found that loss of *sams-1* or *sams-4* has distinct effects on survival during heat stress, gene expression, and histone modification profiles, demonstrating that the enzymatic source of SAM influences its utilization.

Stress responses can also originate from internal sources, such as membrane stress triggered by imbalances in phospholipid levels or alterations in acyl chain desaturation. SAM derived specifically from *sams-1* induces membrane stress by impairing the production of the methylated phospholipid PC. This stress response is distinct from ER-derived lipid bilayer stress and disrupts the cycling and Golgi localization of the GTPase ARF-1. Using TEM, we find that Golgi ministack structure is also lost. We previously showed that loss of Golgi integrity promotes proteolytic maturation of the lipogenic transcription factor SBP-1/SREBP1. More recently, we found that maturation of another ER-resident transcription factor, LET-607/CREBH, is similarly stimulated by low PC, leading to activation of *warf-1/arf-1.1*. WARF-1 accumulates on Golgi puncta under low PC conditions, replacing ARF-1.

Interestingly, low PC-induced LET-607 maturation occurs in the intestine but not the hypodermis, where ER stress regulators appear to play a greater role in its regulation. Our RNA-seq experiments also reveal distinctions between Golgi and ER stress gene expression programs following PC depletion. Taken together, our work highlights the distinct ways metabolism and stress responses interact to regulate transcriptional programs. Understanding how SAM production influences cellular methylation pathways will provide insight into the integration of nutritional and metabolic processes with molecular pathways regulating stress, aging, and lipogenesis.

30 Discovery of genetic suppressors of frataxin deficiency

Joshua D Meisel[1], Pallavi R Joshi[2], Amy N Spelbring[3], Hong Wang[2], Sandra M Wellner[2], Presli P Wiesenthal[2], Maria Miranda[2], David P Barondeau[3], Gary Ruvkun[2], Vamsi K Mootha[21]Biology, Brandeis University, [2]MGH/Harvard Medical School, [3]Texas A&M University

Friedreich’s ataxia (FA) is the most common inherited ataxia and results from a deficiency in frataxin, an allosteric activator of mitochondrial iron-sulfur cluster (ISC) biosynthesis. We previously reported that *C. elegans* lacking frataxin/FRH-1 protein can be rescued by incubation in hypoxia due to an increase in ISC levels. Here, we conducted an unbiased, forward genetic screen in *C. elegans* using permissive and non-permissive oxygen tensions to discover suppressor mutations that bypass the need for frataxin. All mutations act dominantly and are in the ferredoxin *fdx-2* or in the cysteine desulfurase *nfs-1* genes, resulting in amino acid substitutions at the FDX-2/NFS-1 binding interface. Normally FDX-2 and frataxin compete for the same binding site on NFS-1 during ISC biosynthesis, but in the absence of frataxin FDX-2 is bound constitutively to NFS-1. Our *fdx-2* suppressor mutations weaken this interaction; conversely, excess FDX-2 inhibits frataxin-stimulated NFS-1 activity in vitro and blocks ISC synthesis in human cells. Lowering levels of wild-type FDX-2 rescues the growth of frataxin mutant *C. elegans* or the ataxia phenotype of a mouse model of FA. Our work nominates restoring the stoichiometric balance of frataxin and FDX-2 via knockdown of FDX-2 as a potential therapy for FA.

31 A balancing-act mechanism to maintain precise transcription factor levels and neuronal identity in adult *C. elegans* motor neurons

Honorine Destain[1], Paschalis Kratsios[21]Molecular Genetics and Cell Biology, University of Chicago, [2]Neurobiology, University of Chicago

The critical function of transcription factors (TFs) in regulating cell identity depends on their precise levels of expression. TFs function within gene regulatory networks (GRNs), which represent the sum of TF and target gene interactions. While a lot is known about developmental GRNs, GRNs that ensure continuous TF expression at appropriate levels throughout life remain largely unknown. Here, we study UNC-3 (Collier/Olf/EBF), a TF that is continuously required for cholinergic motor neuron identity and *C. elegans* locomotion. Our preliminary data revealed that UNC-3 expression levels are critical for appropriate motor neuron identity. Intriguingly, human genetics studies of EBF3 (UNC-3 ortholog) showed that deviations of normal *EBF3* expression levels lead to a human neurodevelopmental syndrome characterized by motor developmental delay. By identifying the molecular mechanisms that maintain UNC-3 expression in *C. elegans* motor neurons, we may uncover conserved principles applicable to human EBF3, and to other dosage-sensitive TFs. Contrary to classical models of TF positive autoregulation, we propose here a 'balancing-act model' of opposing inputs that maintain UNC-3 expression at precise levels. Positive input is provided by the midbody Hox proteins LIN-39 (Dfd/Scr/Hox4-5) and MAB-5 (Antp/Hox6-8), along with the Hox cofactor CEH-20 (PBX). Strikingly, negative input is provided by UNC-3 itself through negative autoregulation. This negative autoregulation occurs directly, as tested with ChIP-seq and CRISPR/Cas9-mutagenesis of UNC-3 binding sites in the endogenous *unc-3* locus. Temporally controlled protein depletion with the auxin system showed that negative autoregulation is continuous throughout life. Importantly, impaired negative autoregulation results in increased adult worm swim speeds, likely due to increased UNC-3 levels and misregulation of motor neuron identity. To understand how UNC-3 self-represses, we conducted protein domain deletion rescue assays and uncovered a requirement for the N-terminal domain for UNC-3's repressive function. Using an *in silico* protein interaction screen (Colabfold), we identified *set-4,* a histone H4K20me methyltransferase, as a potential cofactor that interacts with the UNC-3 N-terminal domain to mediate repression. Altogether, we propose that this 'balancing-act' model, where combined activating and repressing inputs regulate TF levels over time, could function across long-lived cell types to ensure cell fate maintenance.

32 The importance of transcription rates for embryonic cell fate specification

Priya Sivaramakrishnan Center for Computational and Genomic Medicine, Children's Hospital of Philadelphia/University of Pennsylvania

The developmental transcriptome is highly dynamic with rapid changes in gene expression driving cell fate decisions. In the *Caenorhabditis elegans* embryo, early cell cycles are as short as 15 minutes, making temporal constraints on transcription particularly severe. To determine how quickly the embryonic transcriptome is changing, we estimated transcription rates in the *C. elegans* embryo by combing single- cell RNA sequencing (sc-RNA seq) and single molecule transcript imaging measurements. We found that transcription factors with important roles in cell fate decisions are in particular transcribed at extremely high rates. To study how such high rates are achieved, we are focusing on the mesodermal (MS) lineage, as it is established early, and several MS fate specification factors have high rates of transcription. For example, *ceh-51*, a transcription factor required for the development of multiple muscle tissue types, is transcribed at 30.2 mRNAs/min. This approaches the steric limit possible for PolII transcription. To understand how rates are regulated and their role in fate specification, we are looking at *cis*-regulatory elements (our results suggest control by multiple redundant promoter elements), transcription elongation factors (PolII elongation reduces total rate) and cell size (larger embryos have higher rates). In most organisms, rates are known to scale with temperature. Interestingly, some MS lineage fate regulators show a temperature sensitive phenotype with more defects at 25°C. We are currently combining scRNA-seq with lineage analysis at different temperatures to understand the complex relationship between transcription rates, cell size and temperature. Overall, our goal is to determine how individual factors come together to fine-tune transcription dynamics and the temporal transcriptional control of embryonic cell fate specification.

33 The role of two terminal selectors on the evolution of left-right neuronal asymmetry

Dylan L Castro[1], Ivan Dimov[2], Heather Carstensen[2], Ray Hong[21]Biology, California State University Northridge, [2]California State University Northridge

In C*aenorhabditis elegans,* one major role of the zinc-finger transcription factor CHE-1 is to promote lateral asymmetry in the ASE gustatory neurons. The left and right ASE neurons asymmetrically express receptor-type guanylyl cyclases (*gcy* genes). The regulation of asymmetrically expressed terminal differentiators involves asymmetrically expressed transcription factors *die-1* and *cog-1*, whose expression is regulated via a double negative feedback loop by the miRNAs *lsy-6* and *mir-273*. However, the *lsy-6* miRNA evolved selectively in the *Caenorhabditis* genus and is absent in the predatory nematode, *Pristionchus pacificus.* How is genetic regulation of asymmetry accomplished without the use of a key regulator? The recent finding that Ppa-CHE-1 is expressed in both AFD and ASE neurons provides an opportunity to address how CHE-1 and another terminal selector, TTX-1, coordinate the patterning of both symmetric and asymmetric neurons. To identify terminal effector markers, we used HCR FISH to identify 3 *gcy* subfamilies that are specifically expressed in the AFD, ASEL, and ASER neurons in *P. pacificus* (represented by *Ppa-gcy-8.1, Ppa-gcy-7.2, Ppa-gcy-22.3,* respectively). Using *Ppa-gcy-22.3p::GFP* as the ASER marker, we conducted a limited forward genetic screen for genes that disrupt the asymmetric ASER expression and isolated a *Ppa-die-1* allele with ASER marker expression in both the ASE neurons. We also found that in *die-1* mutants, the ASEL is not completely transformed into the ASER but instead exists in a hybrid ASER/ASEL state. To test for asymmetric regulation of *Ppa-cog-1*, we made a *cog-1-3'UTR* GFP reporter and found that it is predominantly repressed in the ASEL compared to ASER when asymmetric expression was observed, implicating negative post-transcriptional regulation of *cog-1* in the ASEL. Therefore, to see if lateral asymmetry is under miRNA control in *P. pacificus*, we generated a *Ppa-pash-1* mutant likely to be defective in miRNA biogenesis and found partial overlap of the *die-1* mutant 2xASER phenotype. The genetic regulation of *Ppa-ASE* asymmetry is thus likely under miRNA control. Lastly, we found that the terminal selector for AFD neurons, *ttx-1*, is involved in suppressing ASE fate markers in the AFD from a tripotential state present in embryos. Our results demonstrate a possible independent evolution of genetic mechanisms for patterning the laterality of nervous systems and highlight a network of changing regulatory roles from familiar factors.

34 The LIN-42-MYRF-1 complex drives pulsatile transcription to couple developmental progression and temporal cell fate specification

Peipei Wu[1], Jing Wang[1], Robert Morabito[1], Sophia Vrh[1], David Ritter[2], Ercan Sevinc[2], Christopher Hammell[1]1Cold Spring Harbor Laboratory, [2]New York University

During post-embryonic development in *C. elega*ns, the invariant cell division patterns and stage-specific gene expression programs are intrinsically linked to the molting cycles that define each larval stage. The transitions between these stage-specific patterns are regulated by heterochronic microRNAs, which are expressed in a pulsatile manner, with one transcriptional pulse occurring in each larval stage. The mechanisms that generate these transcriptional patterns remain unknown. Here, we demonstrate that global pulses of heterochronic miRNA expression require a core timing complex consisting of MYRF-1 and LIN-42, the conserved *C. elegans* ortholog of the circadian transcriptional repressor. MYRF-1 is dynamically expressed during each larval stage, binding to upstream regulatory elements in all heterochronic miRNAs and is essential for their transcription. We also show that MYRF-1 binds to conserved regulatory elements throughout the *lin-42* gene and is necessary for rhythmic *lin-42* expression. Finally, by employing genetics, AlphaFold structural predictions, and genome editing, we reveal that the MYRF-1/LIN-42 heteromeric complex forms the central component of a transcriptional/translational feedback loop (TTFL) that directly regulates the expression of *lin-4* and *let-7* family microRNAs. In this system, LIN-42 binds directly to multiple surfaces of MYRF-1, leading to the accelerated turnover of nuclear-localized MYRF-1. By directly modulating the turnover rate of MYRF-1, LIN-42 shapes both the amplitude and duration of heterochronic miRNA expression to ensure proper temporal patterning and developmental robustness.

35 Transcriptional and post-translational control of oscillatory gene expression in development

Simona Ligorio[1,2], Yannick Hauser[1], Georg Kempf[1], Helge Grosshans[1,21]Friedrich Miescher Institute for Biomedical Research, [2]University of Basel

Multicellular organisms depend on precise temporal regulation of numerous events to orchestrate development. In *Caenorhabditis elegans*, post-embryonic development involves four repetitions of a developmental module with variation, the larval stage. Accordingly, thousands of genes accumulate rhythmically peaking once per larval stage, each at a different time yet all synchronized with molts. Previously, we identified six transcription factors as putative core components of the underlying oscillator, or clock, including BLMP-1/PRDM1, whose function and regulation we characterize here. We find that BLMP-1 depletion leads to extended molt duration and skin barrier defects due to extensive dysregulation of oscillatory gene expression. We hypothesized that the E3-ubiquitin-ligase DRE-1/FBXO11, a known regulator of BLMP-1 in other processes, could be important for gene expression oscillations and rhythmic development. Indeed, DRE-1 depletion causes a increased BLMP-1 levels and leads to larval arrest after the first molt. Unexpectedly, when DRE-depletion occurs in a *blmp-1(0)* background, worms complete all four larval stages, despite the fact that *blmp-1(0)* single mutant animals themselves fail to proceed past the first molt. Our results reveal that DRE-1 and BLMP-1 are mutually epistatic and imply the existence of additional DRE-1 targets, whose function is partially redundant to BLMP-1. Our current work focuses on *i)* the identification of additional DRE-1 targets through ubiquitinomic/proteomics and genetic suppressor screens, and on *ii)* the molecular and temporal characterization of the established interaction DRE-1/BLMP-1. Taken together, our data data suggest that post-translational regulation is a crucial component of the *C. elegans* developmental oscillator.

36 Developmental shedding and regrowth of sensory cilia

Rachel Swope[1,2], Irina Kolotuev[3], Maxwell G. Heiman[1,21]Harvard Medical School, [2]Boston Children's Hospital, [3]University of Lausanne

Cilia are dynamic signaling organelles with a diversity of strategies for remodeling, including resorption, decapitation, extracellular vesicle release, and stress-induced shedding. Here, we describe a novel example of cilia remodeling in which the tips of sensory cilia are shed and regrown during developmentally controlled cycles called molts. In *C. elegans*, all cilia are located at the dendrite endings of sensory neurons, where they respond to environmental cues. Some cilia are embedded in the cuticle, an apical extracellular matrix (aECM) that covers the entire animal. The cuticle aECM is shed during four larval molts, raising the question of what happens to cuticle-embedded cilia during molts. Using a panel of fluorescent markers for specific types of ciliated neurons, we observed large fragments of cilia in the molting cuticle, suggesting they had been shed from the neurons. Cilia shedding was specific to neurons with cilia embedded in the cuticle aECM (IL1, IL2, OLQ, OLL, and CEP) and was not seen in other cilia, such as those of the amphid. To better understand the ultrastructural changes that accompany cilia shedding, we obtained AT-SEM images of a molting animal. We found that the cilium appears pinched, as if being severed, at the site where it exits the socket glial cell and enters the cuticle aECM. At the same time, a new cilium appears to be growing into a lumenal compartment of the socket glia ending, which is hugely expanded. Notably, the site of cilia severing is surrounded by an electron-dense ECM structure, presumably secreted by the surrounding socket glial cell. We identified a secreted aECM protein, MAM-5, that is expressed transiently during molts only in socket glia associated with cilia shedding. Remarkably, endogenously tagged MAM-5-sfGFP localizes to the site of cilia severing. Finally, in *mam-5* deletion mutants, we observe severely truncated cilia phenotypes, specifically in cilia that normally undergo shedding. We propose that, during molting, glia secrete an aECM complex including MAM-5 to 'pinch off' the distal tips of sensory cilia and promote regulated regrowth. Cilia shedding could serve to reset cilia functionality between life stages in *C. elegans* and remove damaged cilia after injury or reprogram cilia to adapt to altered environmental conditions in other organisms.

37 *daf-16/FOXO* and *daf-12/NHR* coordinate stage-specific cell fate with dauer arrest and recovery

Matthew J Wirick[1], Amelia F Alessi[2], Himani Galagali[2], Benjamin S Olson[1], Isaac T Smith[1], John K Kim[2], Xantha Karp-
[11]Central Michigan University, [2]Johns Hopkins University

The mechanisms by which developmental pathways are modulated to accommodate developmental arrest are poorly understood. In adverse environments, dauer diapause can interrupt developmental progression after the second larval molt. During continuous (non-dauer) development, a heterochronic molecular timer consisting primarily of microRNAs and their targets controls the progression of stage-specific cell fates in lateral hypodermal seam cells. In unfavorable conditions, the DAF-16/FOXO transcription factor and the DAF-12 nuclear hormone receptor in its ligand-free state promote dauer formation and oppose the expression of *let-7* family microRNAs, thus pausing developmental progression during dauer. Surprisingly, we found that *daf-16(0)* post-dauer adults showed reiterative heterochronic defects including missing or gapped adult alae, lack of seam-cell fusion, and reduced expression of the adult-specific *col-19p::gfp* marker. Consistent with these adult cell fate defects, *let-7*-family microRNA expression was reduced in *daf-16(0)* post-dauer larvae. Addition of the DAF-12 ligand, dafachronic acid, suppressed the reiterative phenotypes in *daf-16(0)* post-dauer animals. Notably, addition of dafachronic acid to *daf-16* mutants rescued levels of *let-7* family transcriptional reporters but did not affect levels of these reporters in *daf-16(+)* control strains. Based on RNA-seq and ChIP-seq data from *daf-16(0)* and control dauer larvae, we hypothesize that *daf-16* regulates levels of dafachronic acid by directly activating the expression of *scl-12* and *scl-13,* genes that are required to sequester cholesterol before dauer entry to allow the production of dafachronic acid upon dauer recovery. Consistent with this hypothesis, timed auxin-mediated depletion of *daf-16(aid)* indicated that *daf-16* is required before dauer formation, rather than after dauer, to prevent reiterative seam cell fates in post-dauer larvae and adults. Taken together, we propose a model whereby as larvae enter dauer, *daf-16* blocks expression of *let-7*-family microRNAs and promotes expression of *scl-12/13,* leading to developmental arrest and sequestration of cholesterol. At the same time, ligand-free DAF-12 promotes dauer formation and further represses the *let-7s.* Upon recovery, sequestered cholesterol is converted to dafachronic acid. Ligand-bound DAF-12 promotes dauer recovery and expression of *let-7* family microRNAs*,* thereby promoting adult cell fate. Thus, *daf-16* and *daf-12* coordinate dauer formation and exit with the arrest and resumption of cell fate pathways.

38 3’ UTR-dependent regulation of *ifet-1* is essential for sperm production and fecundity in *Caenorhabditis elegans*

Rita I Okeke[1], Lu Lu[2], Allison Abbott[31]Biological Sciences, Marquette University, [2]Genetics, University of North Carolina, Chapel Hill, [3]Marquette University

MicroRNAs(miRNAs) are small non-coding RNAs that are key regulators of gene expression; however, their direct mRNA targets involved in sperm development remain largely unknown. *ifet-1*, which encodes an eIF4E binding protein, functions in the germline to repress translation of its targets and is a predicted target of multiple miRNAs. While *ifet-1* is required for oogenesis, its role in spermatogenesis remains unclear. Here, we demonstrate that 3›UTR-dependent regulation of *ifet-1* mRNA is essential for optimal spermatogenesis and embryonic development. Using CRISPR-Cas9, we generated a series of *ifet-1* 3’UTR deletion mutations to identify regions critical for post-transcriptional regulation. Replacing the *ifet-1* 3’UTR with the germline-permissive *tbb-2* 3’UTR resulted in reduced brood size, elevated embryonic lethality, adult lethality, and a 3-fold upregulation of *ifet-1* mRNA levels. These findings suggest that the *ifet-1* 3’UTR is essential for optimal fecundity and development. Mutants with a 152nt deletion showed a 38% decrease in brood size, lower sperm count due to a premature sperm-to-oocyte switch and a 2-fold increase in *ifet-1* mRNA levels. Notably, these mutants lack the *mir-44* miRNA family binding site and exhibit phenotypes comparable to *mir-44* family mutants. Mutational analysis also indicated that a different 116nt region is associated with a delayed spermatogenesis rate. Deletion mutations that remove all but 94nt of the 3’UTR exhibited >45% embryonic lethality, adult lethality, and a reduced brood size with a 3-fold increase in *ifet-1* mRNA levels. For these mutants, immunofluorescence revealed abnormal IFET-1 localization in embryos and loss of perinuclear localization in the L4 germline. Our findings suggest a complex regulatory network that is likely to involve microRNAs and possibly RNA binding proteins function through the 3› UTR of *ifet-1* to govern sperm production, fecundity, and embryonic development. Ongoing analyses aim to identify specific microRNAs that contribute to the regulation of *ifet-1* in the germline, providing better insights into the molecular roles of miRNAs in *C. elegans* sperm development and reproductive success.

39 FBFs cooperate with small RNAs in *C. elegans* germline stem and progenitor cells

Mary Ellenbecker, Gabriella Weiss, Camille Spencer, Elizabeth Sweeney, Ekaterina Voronina Division of Biology, University of Montana

Pumilio-family RNA-binding proteins FBF-1 and FBF-2 are well-known regulators of *C. elegans* germline stem cell maintenance (Zhang et al.,1997; Crittenden et al., 2002). Previously, we found that FBF proteins localize within or adjacent to P granule compartment of the germ granules, the non-membrane-bound perinuclear organelles essential for fertility. *C. elegans* germ granules serve as hubs of small RNA metabolism and support the transgenerational inheritance of small RNA regulation, with specific germ granule compartments performing distinct regulatory functions (Phillips and Updike, 2022).

We hypothesized that localization of FBF proteins to germ granules reflects their involvement in small RNA-dependent regulation. Consequently, we predicted *fbf* mutations would disrupt small RNA-mediated processes. Supporting this hypothesis, we found that both *fbf-1* and *fbf-2* mutants are defective in transgenerational inheritance of RNAi targeting a germline GFP reporter. Specifically, both mutants showed accelerated re-expression of GFP in progeny removed from parental RNAi exposure. Notably, the initial RNAi response in the parental generation remained unaffected. Further analysis revealed impaired production of pUG-ylated intermediates of secondary siRNA biogenesis, indicating that the RNAi inheritance defect results from a failure of siRNA amplification. Because disruption of RNAi inheritance often correlates with abnormalities in germ granule compartments, we investigated germ granule structure in *fbf* mutants. We observed abnormal cytoplasmic aggregates of MUT-16::GFP, a core component of *Mutator* foci, in the distal germlines of *fbf* mutants. Surprisingly, this mislocalization of MUT-16 resolved by the pachytene, where the typical organization of P granules and *Mutator* foci recovered.

Taken together, our findings reveal a novel role for FBF proteins in small RNA-mediated regulation of gene expression in *C. elegans*. FBFs contribute to proper organization of germ granule subcompartments in the distal germline. Furthermore, effective transgenerational RNAi inheritance requires functional *Mutator* foci in the distal germline. This may reflect the necessity for reinitiating sRNA amplification in the stem and progenitor cells to be maintained in the bulk of the germline.

40 Nuclear RNAi machinery promotes direct reprogramming of somatic cells

Gizem Köse[1], Ismail Özcan[1], Anna Reid[2], Margaux Quiniou[2], Baris Tursun[11]Molecular Cell Biology Unit, Institute of Cell and Systems Biology of Animals, University of Hamburg, [2]University of Hamburg

Cellular reprogramming is a promising strategy for future tissue replacement therapies. Understanding the gene regulatory pathways of cell fate conversion is central to ensuring the function and safety of reprogrammed cells. We discovered that coelomocytes (CCs), which have scavenging and hepatic function in *C. elegans,* can be directly reprogrammed to intestinal cells that acquire a unicellular lumen and microvilli or neuronal-like cells with corresponding morphology and gene expression properties. Direct reprogramming (DR) of CCs is induced by overexpression of the transcription factor ELT-7 (intestine) or CHE-1 (ASE neuron) and is accompanied by loss of CC-related gene expression and physiological functions. An RNAi screen for enhancers and suppressors of DR identified numerous chromatin-regulating factors. For instance, depletion of H3 and H4 core histone genes or components of the conserved SET1/MLL complex enhances CC reprogramming. Conversely, the depletion of specific Argonauts (AGOs), key effector proteins in small-RNA-guided gene-regulatory pathways, suppresses DR of CCs. Additional members of small RNA pathways, such as SAGO-1/2 and the RDE-10/11 complex, were also identified to be required for efficient DR. NRDE-3, the core AGO of the nuclear RNAi machinery, shows the most robust suppression in CC reprogramming upon its depletion. At the same time, its overexpression increases the efficiency of CC to ASE neuron conversion. Furthermore, we provide data suggesting cooperation of NRDE-3 with the Histone methyltransferases SET-25 and MET-2 that deposit the repressive chromatin modification H3K9me3. Overall, the nuclear RNAi machinery axis via NRDE-3 and SET-25 / MET-2 promotes DR of CCs – an unexpected finding as repressive chromatin mechanisms usually act as reprogramming barriers. Our findings may indicate a general dichotomous context dependency of chromatin regulatory mechanisms during different cell fate conversion phenomena.

41 How is the Number of Natural Cell Reprogramming Events Restricted in *C. elegans*?

Jeanne Cury IGBMC, University of Strasbourg

While most cells keep their identity all along their life, some cells have the capacity to change identity, a phenomenon called cellular plasticity. A special case is transdifferentiation (Td), where a differentiated cell directly converts into another differentiated cell type. Despite its therapeutic potential, Td remains poorly understood.

The team uses the worm *C. elegans*, which is particularly suitable for studying Td *in vivo* due to its natural Td events, small number of cells, and well-known, invariant lineage, making it an innovative and robust model to study natural cell plasticity at the single-cell level.

To understand why some cells can naturally undergo Td ***in vivo*** while others cannot, my study focuses on the Y-to-PDA Td, where a rectal cell called "Y" naturally transdifferentiates into a neuron called "PDA", a robust phenomenon present in 100% of worms during their development. Among the surrounding cells, only the Y cell undergoes Td into a PDA neuron. To identify mechanisms that could control the number of cells capable of Td, we examined mutant contexts with extra Y-to-PDA Td events. By doing so, we described two mechanisms:

A positive selection of the cell capable of Td by the Notch signaling (Daniele *et al.,* 2025): we found that a short Notch signal promotes both the Y identity and its ability to transdifferentiate. When ectopically-activated, Notch is also sufficient to provoke the formation of an extra competent Y cell that will transdifferentiate into a PDA neuron.

A negative selection by ***hlh-14***: in absence of *hlh-14*, an ortholog of Ascl1 (a key proneural transcription factor used in *in vitro* reprogramming experiments) an additional Td occurs. We found that this transcription factor blocks the formation of extra Td events by over-imposing a neuronal fate (DA9 neuron).

In addition, these two selection mechanisms do not act independently: Notch inhibits the expression of *hlh-14* to induce a Y identity and its ability to reprogram.

Furthermore, we identified a third process that negatively regulates the number of Td. Surprisingly, this mechanism occurs in yet another different cell in the rectal area and is likely mediated by an additional negative mechanism, independent of Notch and *hlh-14*.

In conclusion, our work reveals that different cells are capable of Td, and that there are several mechanisms, in different cells, limiting this process. It sheds a light on the complexity of the barriers to be overcome to be able to produce in situ replacement cells by Td (regenerative therapies).

42 Sexually dimorphic Argonaute structure and localization facilitate sex specificity of small RNA pathways in *C. elegans* germ cells.

Acadia L DiNardo[1], Nicole A Kurhanewicz[1], Hannah R Wilson[1], Elena Kuypers[2], Rachael Giersch[1], Diana Libuda[11]Institute of Molecular Biology, University of Oregon, [2]Biology, University of Oregon

Germ cell proliferation and proper genome inheritance are critical to maintain fertility through generations. To promote proper germ cell development and fertility, small RNA pathways employ Argonaute proteins (AGOs) to downregulate aberrant transcripts. In *Caenorhabditis elegans,* two of these AGOs, WAGO-1 and WAGO-4, localize to the germ granule, a cytoplasmic, phase separated compartment adjacent to nuclear pore complexes of germ cells. While previous studies have found that WAGO-1 and WAGO-4 regulate distinct pools of genes in a sex-specific manner, the mechanism by which WAGO-1 and WAGO-4 achieve sexually dimorphic gene regulation is largely unknown. Here we identify sexual dimorphisms in WAGO-1 and WAGO-4 localization and function of protein structural features that affect their sex-specific transcript regulation during *C. elegans* germ cell development. During meiotic prophase I progression, we find that the structural germ granule components (PGL-1, PGL-3, and ZNFX-1) and the PIWI-homolog PRG-1 display dynamic and distinct localization patterns between egg and sperm development that elicit differential WAGO-1 and WAGO-4 localization and biophysical properties. Only during spermatogenesis, PGL-1, ZNFX-1, and WAGO-1 are encompassed within a shell of PRG-1 and a ring of PGL-3. These striking protein organizations encircling the WAGO-containing compartments of the germ granule suggest sex-specific roles of PGL-3 and PRG-1 in regulating germ granule structure. Further, we identify sexually dimorphic functions of specific WAGO-1 and WAGO-4 structural features. Disruption or modifications of the intrinsically disordered N-terminus (IDR) of WAGO-1 and WAGO-4 causes loss of PGL-1 phase separation only during spermatogenesis. MDAnalysis of the WAGO-1 and WAGO-4 protein structures reveal that germ granule disruptions are likely due to prolonged association of the modified IDR within the WAGO-RNA binding pocket. In addition, deletion of the MID and PIWI-domains of WAGO-1 causes complete sterility only in males. Finally, we reveal sexually dimorphic interdependence of WAGO-1 protein abundance on WAGO-4 structure. Together, our studies suggest sexual dimorphisms in the structure and stability of the germ granule may drive sex specific roles of Argonaute gene regulation during germ cell development.

43 Locked or flexible? Heterogeneity in the plasticity of sexual state in the *C. elegans* nervous system

Jiarui Zhang[1], Carlos A. Diaz-Balzac[2], Douglas S. Portman[1,3,41]Department of Biology, University of Rochester, [2]Department of Medicine, Endocrine/Metabolism, University of Rochester (SMD), [3]Department of Biomedical Genetics, University of Rochester (SMD), [4]Department of Neuroscience, University of Rochester (SMD)

Sexual differentiation is often seen as irreversible, with sex-specific morphology and behavior developmentally locked in adulthood. But is this truly the case? In *C. elegans*, biological sex is dictated by the master regulator *tra-1*, a transcription factor that is necessary and sufficient to repress all male-specific characteristics in the soma. In addition to overt sexual dimorphisms, sex-specific patterns of gene expression in sex-shared neurons are also under *tra-1* control. This could occur via two mechanisms: *tra-1* might irreversibly implement the hermaphrodite state by acting transiently during development, or it might be required continuously to maintain sex-specific gene expression patterns in adults. To investigate this, we used the auxin-inducible degron system to remove *tra-1* function specifically from the nervous system. We examined the effects of this on several markers of sexual differentiation, including *srj-54*, a GPCR male-specifically expressed in the AIM interneurons, and *odr-10*, a food chemoreceptor that is male-specifically repressed in the AWA sensory neurons. As expected, degrading TRA-1 in the nervous system specifically of the XX individuals throughout development ectopically activated *srj-54* in AIM and repressed *odr-10* in AWA. Next, we induced TRA-1 degradation at specific developmental stages. In AIM, we identified a critical window for *tra-1* function: loss of TRA-1 before or during the L3 stage activated *srj-54*, while removing TRA-1 in adults left the hermaphrodite state intact. This suggests that TRA-1 irreversibly programs AIM's sexual state during development. In AWA, however, *tra-1* appears to act differently: removal of *tra-1* either during development or in adulthood was sufficient to repress (i.e., masculinize) *odr-10* expression, suggesting that the sexual state of AWA is continuously dependent on *tra-1*. This distinction may be related to neuronal function: AIM, an interneuron, could irreversibly commit to a sexual state during the juvenile-to-adult transition, whereas AWA, a sensory neuron, remains plastic to allow greater behavioral flexibility. Our findings provide new insight into the temporal regulation of sex-specific gene expression, revealing unexpected plasticity and heterogeneity in the permanence of sexual state in the *C. elegans* nervous system. Future work will explore the extent to which sexual state is dynamic in adults and the mechanisms underlying the cell-type specificity of *tra-1* function.

44 Uncovering translation regulation during *C. elegans* development using cell-type specific ribosome profiling

Loes Steller[1], Alexander Blackwell[1], Suzan Ruijtenberg[21]biology, Utrecht University, [2]Biology, Utrecht University

Protein synthesis is a key step in decoding the information stored in our genome. Consequently, translation regulation plays a crucial role in shaping the cellular proteome, thereby determining cell fate and function. The development of ribosome profiling – genome-wide sequencing of small RNA fragments that were bound by a single ribosome - provided valuable insights in i*n vivo* translation rates. However, identifying cell-type specific changes in translation is often challenging, due to the requirement of substantial amounts of starting material. To address this, we developed a low-input ribosome profiling method for *C. elegans*, which allows us to track cell-specific dynamics of translation regulation throughout development. We obtained high quality translation profiles from a time series of experiments using as little as 100 synchronized *C. elegans* mesoblast cells per timepoint.

Analysis revealed widespread and dynamic changes in both the transcriptome and translatome as cells progress from a multipotent progenitor to fully differentiated muscle cells. The majority of differentially expressed genes are regulated at the level of transcription. However, we also identified genes regulated exclusively at the level of translation and genes where changes in translation intensify or buffer transcriptional changes. Furthermore, we find co-regulated gene clusters with similar functions (including muscle differentiation and cell cycle genes), possibly indicating shared regulatory mechanisms. Taken together, low-input ribosome profiling reveals how multilayered regulation of gene expression shapes the proteome throughout development. Moreover, this technique could be extended to other cell types, thereby broadening its utility in uncovering how changes in translation efficiency affect organismal behavior.

45 The sequence requirement for DNA double-strand breaks and neotelomere formation during programmed DNA elimination in *Oschieus tipulae*

Jansirani Srinivasan[1], Marro Agbaga[1], Vincent Terta[1], Brandon Estrem[1], Thomas Dockendorff[1], Jianbin Wang[21]The University of Tennessee Knoxville, [2]Biochemistry & Cellular and Molecular Biology, The University of Tennessee, Knoxville

Programmed DNA Elimination (PDE) is an exception to genome integrity. It specifically removes selected DNA sequences during development. An increasing number of species are being discovered to undergo PDE, including many free-living nematodes, suggesting PDE is biologically significant. However, the functions and mechanisms of PDE in multicellular organisms remain largely unknown. Using nematode *Oscheius tipulae* as a model organism, we are investigating the molecular mechanisms of the DNA double-strand breaks (DSBs) that fragment the chromosomes. *O. tipulae* eliminates 0.6% of its 60-Mb germline genome, with 12 DSBs occurring at both ends of all six chromosomes. After DSBs, the chromosome ends are capped with newly synthesized telomeres. We identified a 30-bp degenerated palindromic motif, termed Sequence For Elimination (SFE), at the sites of DSBs. Mutants with disrupted SFE motifs prevented PDE at the modified sites, confirming that SFE is necessary for PDE. Interestingly, replacing a wild-type SFE at chromosome II left end (chrII-L) with the consensus sequence of the SFE, which does not exist in the *O. tipulae* genome, successfully induced PDE. This suggests a trans-acting factor is likely used to recognize the SFE motif. Additional mutants within the consensus SFE motif further elucidated the functional regions of the motif. Notably, the highly conserved GGC within the SFE matches the telomere sequence TTAGGC, suggesting it may have a function in telomere healing. Using a mutant that changes the conserved GGC to GCC but maintains the motif's ability to perform PDE, we determined a priming role of the GGC sequence for the neotelomere formation. Furthermore, mutants that introduced the SFE consensus at locations 1,000 bp and 10,000 bp away from the original chrII-L site were sufficient to induce PDE, demonstrating that the motif can function independently at a new site. Efforts are underway to introduce the SFE in the middle of a chromosome to see if the SFE can break the chromosome and lead to karyotype change. Overall, our data provide mechanistic insights into the DSBs and neotelomere formation processes essential for nematode PDE.

46 Genes have expression ceilings that predict overexpression sensitivity

Swetha Iyer, Sophie Sanchez, Brooke Frohock, James Groh, Annika Agnihotri, Eva Beckett, Caymee Bigham, Ella DeMott, Ansley Fiorito, Reece Jones, Nina Mourao, Katherine Perks, Sofia Smith, Briana Syed, Jon Pierce University of Texas at Austin

Many genetic disorders are caused by gene duplication (e.g. Down syndrome), but it is hard to know which genes are sensitive or tolerant to duplication in part because most organisms tolerate extra copies of many genes. To identify factors that predict gene duplication sensitivity, we transformed 47 orthologs of human 21st chromosome genes one at a time in *C. elegans*. More than half caused developmental, behavioral, or reproductive deficits as extra copies. We used logistic regression to help identify factors that predict gene overexpression sensitivity. Interestingly, most overexpression-sensitive genes displayed a ceiling in expression across wild *C. elegans*, as though individuals with higher levels are selected against in nature. This model extended to over a dozen independent worm studies including those on genes that cause overexpression phenotypes when derepressed in microRNA mutants. The model also generalized to humans. We found that duplication-sensitive genes that cause severe medical conditions display a ceiling of expression in a healthy population, e.g. *APP* - Alzheimer's disease, *PCSK9* – hypercholesterolemia*, PMP22* - Charcot-Marie-Tooth disease type 1A, and *SNCA* – Parkinson's disease. Likewise, about half of 1,048 genes contained within duplication-sensitive copy-number variant regions also display expression ceilings. Conversely, the vast majority of 1,029 example duplication-tolerant genes do not display expression ceilings – with intriguing exceptions. To quantitatively test our ceiling hypothesis, we have begun testing if expression of seven genes just above or below their expression ceiling results in overexpression phenotypes in worm. So far, our hypothesis appears to hold where expression needs to surpass the ceiling threshold to cause an overexpression phenotype. Additionally, we are developing computational methods to further identify genes with hard vs soft ceilings, search for expression floors to predict haploinsufficient genes, and study combinatorial gene expression interactions for worm and human genes. Altogether, our model suggests that the presence and value of an expression ceiling observed in healthy members of a species predicts if, and at what level, a gene may be sensitive to cause an overexpression phenotype.

47 SUMOylation of Chromodomain Protein MRG-1 Regulates Cell-Fate Specification

Johan Girgenrath[1], Craig C. Mello[21]RTI, UMass Chan Medical School, [2]Umass Chan Medical School

MRG-1, a conserved chromodomain protein, regulates gene expression by binding methylated histone H3K36. It promotes germline gene expression while repressing transgenes and X-linked genes in *C. elegans*. MRG-1 interacts with both histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes, suggesting it recruits opposing chromatin-remodeling factors in a context-dependent manner. However, the mechanism underlying this regulation remains unclear.

We identify SUMOylation at lysine 301 (K301) as a key post-translational modification of MRG-1. *mrg-1(K301R)* mutants, which lack SUMOylation, exhibit increased sensitivity to NuRD complex depletion, leading to ectopic germline gene expression in somatic tissues and developmental arrest. Unlike *mrg-1* null mutants which suppress the larval arrest and soma-to-germline cell fate transformations caused by NuRD complex depletion, *mrg-1(K301R)* has the opposite effect enhancing the arrest phenotype while increasing the ectopic expression of germline genes in somatic tissues.

In contrast, during later development *mrg-1(K301R)* behaved similarly to *mrg-1* loss of function, suppressing the synthetic multivulval (*synMuv*) phenotype of *lin-15AB* mutants, *and* decreasing ectopic *lin-3* expression in hypodermal cells.

Immunoprecipitation (IP)/MudPIT analysis reveals that SUMOylation modulates MRG-1's interactions with chromatin regulators: *mrg-1(K301R)* preferentially associates with the Sin3S HDAC complex, while wild-type MRG-1 interacts more with the MYST HAT complex. These findings suggest that SUMOylation directs MRG-1's recruitment of chromatin-remodeling complexes, influencing cell-fate decisions in distinct developmental contexts.

These findings suggest that while MRG-1 is required for proper germline gene expression, its SUMOylation in adults and/or early embryos promotes NuRD complex functions necessary for the transition from germline to somatic fates. In early development, loss of SUMOylation enhances NuRD depletion phenotypes, leading to increased ectopic germline gene expression in somatic tissues and developmental arrest. However, during later development, both MRG-1 function and its SUMOylation are required in synMuv mutants to prevent ectopic vulval specification, likely through regulation of *lin-3* expression through recruitment of MYST complex components. Thus, SUMOylation serves as a critical regulatory mechanism that enables MRG-1 to differentially modulate chromatin-remodeling activities in distinct developmental contexts.

48 Ectopic transcription due to inappropriately inherited histone methylation may interfere with the ongoing function of terminally differentiated cells

Monica Reeves[1], Juan Rodriguez[1], Liyang Sun[1], Karen Schmeichel[2], David Katz[11]Emory University, [2]Oglethorpe University

At fertilization, histone modifying enzymes drive massive epigenetic reprogramming that is vital for appropriate embryonic and postnatal development. In *C. elegans*, the H3K4 demethylase SPR-5/LSD1/KDM1A and the H3K9 methyltransferase MET-2/SETDB1 act together as a repressive switch during this reprogramming, preventing the inappropriate inheritance of histone methylation and the inappropriate maintenance of transcription. Progeny of *spr-5; met-2* double mutants have a severe chemotaxis defect caused by the ectopic expression of germline genes in somatic tissues, but the mechanism through which inappropriate transcription impairs behavior remains unclear. Here we take advantage of the invariant lineage and simple invariant nervous system of *C. elegans* to address this question. Despite finding by single-cell RNAseq that germline genes are already widely ectopically expressed in *spr-5; met-2* 200-cell embryos, we observed that *spr-5; met-2* mutants have almost no somatic lineage defects up to the 350-cell stage of embryogenesis. In addition, NeuroPAL analysis confirmed that adult *spr-5; met-2* mutant worms have a complete nervous system, with 302 neurons. This raised the possibility that the altered chemotaxis behavior may be due to an ongoing defect in terminally differentiated cells rather than a defect in the development of the nervous system. To directly test this question, we used RNAi against the germline transcription factor LSL-1 to shut off the ectopic expression of germline genes in L2 *spr-5; met-2*. Strikingly, we find that suppressing ectopic germline expression rescues normal chemotaxis behavior in the same adult worms that previously exhibited a chemotaxis defect at the L2 stage. This suggests that ongoing ectopic transcription can disrupt normal behavior in a fully intact nervous system. Human patients with corresponding mutations exhibit craniofacial defects, developmental delay, intellectual disability and behavioral abnormalities. Our data raise the possibility that the intellectual disability and altered behavior in these patients could be driven by ongoing ectopic transcription and may be reversible.

49 CAGE-FC: An Accessible Tool for Optical Control of Gene Expression with Single-Cell Resolution

Jose Vazquez, Sebastian Greiss University of Edinburgh

Genetic code expansion (GCE) technology allows for the co-translational incorporation of amino acids beyond the canonical 20 found in nature. Such non-canonical amino acids (ncAAs) can impart new functionalities to existing proteins, providing novel approaches to study biological processes. Examples include ncAAs carrying bioorthogonal handles to site-specifically attach labels or other functional groups via click chemistry, and photo-caged amino acids, which allow for the design of light-activatable proteins.

GCE is achieved using aminoacyl-tRNA synthetase (aaRS)/tRNA pairs that are orthogonal (i.e. do not interact) with their endogenous counterparts. The orthogonal aaRS attaches the ncAA to its cognate tRNA and then the aminoacylated tRNA decodes a designated mRNA codon, usually a UAG stop codon, to incorporate the ncAA into the protein of interest.

Using GCE, Cre and FLP can be rendered photo-activatable by replacing critical residues with photo-caged variants. The caging group of the photo-caged ncAA blocks the activity of the recombinase, until it is removed by brief exposure to long wave UV light, to yield the active wild-type recombinase.

We have previously used GCE to separately express photo-activatable variants of FLP and Cre in *C. elegans*. Using a 365 nm microscope-mounted laser, the activating light could be precisely targeted, granting single-cell resolution to the control of gene expression.

We have since improved this method by establishing a recombinase system that can be activated with 405 nm light, enabling the control of target genes with single-cell resolution using a standard confocal microscope laser.

Furthermore, we have expanded on the photo-activatable recombinase toolkit by developing CAGE-FC (photoCaged-ncAA mediated optical control of Gene Expression – using FLP and Cre). CAGE-FC combines novel photo-activatable variants of FLP and Cre for independent optical control of two target genes in the same animal.

To accomplish this, we have established the concurrent incorporation of two ncAAs in the same animal using mutually orthogonal aaRS/tRNA pairs. Introducing photo-caged ncAAs with distinct absorption spectra then allows us to use two different wavelengths to activate Cre and FLP and thus selectively control the expression of two target genes, with each wavelength controlling the expression of one gene.

CAGE-FC, for the first time, makes possible the independent spatiotemporal control of two target genes in *C. elegans*.

50 tRNA fragments, how 30-40 nucleotides impact fertility and development

Blair K Schneider[1], Nicholas S Galambos[2], Colin C. Conine[1,3]1Neonatology, Children's Hospital of Philadelphia, [2]biology, University of Pennsylvania, [3]Pediatrics (Neonatology & Newborn Services), Perlman School of Medicine at the University of Pennsylvania

Darwin's principle of survival of the fittest relies on parents' ability to pass traits to offspring that support survival and reproduction. While adaptative phenotypes are typically inherited through genomic variation, it has recently become appreciated that epigenetic information in gametes can also communicate environmentally responsive adaptations. One molecule shown to transmit non-genetically inherited phenotypes regulated by the environment are small RNAs. It has been demonstrated in mice that paternal diet influences levels of a novel type of small RNA, tRNA fragments (tRFs), which are nucleolytic cleavage products of tRNAs. Further, in this system tRFs in sperm regulate post-fertilization embryonic gene expression and casually transmit phenotypes to progeny. How tRFs are generated in the germline, and the molecular mechanisms underlying gene regulatory functions of tRFs remain unknown. We identified a nuclease, *rnst-2*, in *C. elegans* which regulates the accumulation of tRFs in sperm. Further, we found that these tRFs regulate post-fertilization embryonic gene expression and phenotypes of progeny. To expand upon tRF biology in *C. elegans* I aim to determine new components of tRF biogenesis and the functions underlying their regulatory ability. For example, I have identified suppressor mutations of a specific aspect of the rnst-*2(null)* phenotype, hatching and larval development, which we have mapped to several interesting candidate genes involved in RNA metabolism. Further, I have found that the exonuclease *disl-2* has an impact on tRF biogenesis in both the male and female germlines, unlike *rnst-2* which functions specifically in the male germline to process tRFs. To assess how tRFs functionally impact gene expression, I have performed small RNA and mRNA sequencing on *rnst-2* mutant embryos and L1 larvae. Interestingly, these findings have revealed that distinct tRFs accumulate at different developmental stages to uniquely regulate developmental gene expression. These findings establish *C. elegans* as a powerful model to study tRF biology throughout an organism and during development. Importantly, using the worm to study tRF biology is revolutionizing our understanding of how tRFs are generated, regulate gene expression in different tissues and development stages, and perhaps most interestingly transmit non-genetically inherited phenotypes across generations.

51 RNA Pol I maintains the H3K4me3 gradient and chromatin organization required for oogenesis

Raquel Mejia-Trujillo, Qiuxia Zhao, Elif Sarinay Cenik Molecular Biosciences, University of Texas at Austin

This study uncovers a non-canonical, ribosome independent role for RNA Polymerase I (Pol I) in regulating chromatin dynamics during oogenesis in *Caenorhabditis elegans*. Using the auxin-inducible degron system, we selectively depleted a catalytic subunit of RNA Pol I and a ribosome assembly factor, allowing us to distinguish the effects of nucleolar disruption from those of reduced ribosome biogenesis. While ribosome assembly disruption minimally impacted oocyte production, loss of nucleolar integrity via RNA Pol I depletion led to pronounced meiotic chromatin disorganization, and fewer yet significantly larger oocytes. This phenotype coincided with increased chromatin accessibility at oogenesis-specific promoters, enriched for EFL-1 binding sites. These promoters are also correlated with H3K4me3 domains canonically remodeled during the germ-to-oocyte transition. Consistent with these observations, Pol I depletion dampened the distal-proximal H3K4me3 gradient through premature deposition of this histone mark, which is essential for shaping oocyte chromatin architecture. Together, our findings reveal that RNA Pol I activity and nucleolar integrity play a critical epigenetic role during germ cell maturation, independent of ribosome production. Given the evolutionary conservation of nucleolar integrity and H3K4me3 remodeling in female gametogenesis, these results suggest that similar nucleolar functions may play a critical role in fertility, particularly under nucleolar stress, caused by environmental factors, aging, or genetic disorders.

52 Inappropriate inheritance of histone methylation perturbs muscle structure and function

Josh Labus[1], Hiroshi Qadota[2], Guy Benian[2], Penelope Rodriguez[1], Mattie Villhauer[1], Brandon Carpenter[1]1Cellular and Molecular Biology, Kennesaw State University, [2]Department of Pathology, Emory University

Transcription-coupled histone methylation acquired at sperm and oocyte genes during gametogenesis helps establish distinct gamete cell fates. Like DNA, acquired histone methylation can be inherited between generations and must be maternally reprogrammed at fertilization to reset the epigenetic ground state of the zygote. During maternal reprogramming of histone methylation in *C. elegans*, H3K4me1/2 is removed by the H3K4 demethylase, SPR-5, and H3K9me1/2 is subsequently added by the histone methyltransferase, MET-2. Maternal reprogramming by SPR-5 and MET-2 is antagonized by the H3K36 methyltransferase, MES-4, which maintains H3K36me2/3 at germline genes to ensure proper germline gene expression in germ cells. In the absence of SPR-5; MET-2 maternal reprogramming MES-4 maintains H3K36me2/3 at germline genes in the soma leading to somatic expression of germline genes and abnormal developmental phenotypes. In addition to MES-4 germline genes, a recently identified germline transcription factor, LSL-1, is also misexpressed in spr-5*; met-2* mutants and may contribute to maintaining germline gene expression in the soma of these mutants. Here, we explore how misinherited histone methylation affects specific tissues by examining muscle morphology and function in early, middle and late generation *spr-5* and *met-2* single mutants that inherit increasing levels of aberrant H3K4me2. Interestingly, *met-2* mutants show lower motility levels compared to wildtype and *spr-5* mutants at early generations, but this initial decrease in motility does not decline over generations, even as muscle sarcomeres become moderately disorganized. In *spr-5; met-2* mutants we find that muscle sarcomeres and motility are severely compromised and that maternal knockdown of either MES-4 or LSL-1 in *spr-5; met-2* mutants rescues these muscle defects. Despite the muscle defects that we observed in *spr-5; met-2* mutants, muscle specific gene expression is normal. Finally, we show that muscle cells overexpressing low levels of LSL-1 display sarcomere disorganization. Together, our findings provide a unique insight into how tissue specific phenotypes arise when histone methylation is inappropriately inherited and support a model where germline expressed proteins themselves may perturb somatic cell function without compromising the normal somatic transcription program.

53 Exploring post-transcriptional regulation of gene expression throughout *C. elegans* embryonic development

Felicia Peng[1], C Erik Nordgren[2], John I Murray[1]1Genetics, University of Pennsylvania, [2]University of Pennsylvania

During development, cells undergo dynamic changes in gene expression that are required for appropriate cell fate specification. Although both transcription and mRNA degradation contribute to gene expression dynamics, patterns of mRNA decay are less well understood. Studies of maternal and zygotic mRNA decay have established that transcript stability is largely regulated by the binding of protein or RNA factors to *cis*-regulatory elements within the 3′ untranslated region of transcripts. Codon usage is another major determinant of mRNA stability, as translation can affect transcript stability in a codon-dependent manner. We hypothesize that the regulation of zygotic mRNA decay is complex and contributes to appropriate cell fate decisions throughout embryonic development. To explore this, we examined mRNA decay rates during embryogenesis in *Caenorhabditis elegans*. By sequencing embryonic cells treated with a transcription inhibition time course at both the bulk and single cell level, we measured mRNA half-lives globally in embryos. We identified transcript features that are correlated with mRNA stability and found that mRNA decay rates are associated with distinct peaks in gene expression over time. Moreover, we found evidence that, on average, mRNA is more stable in the germline than in the soma and in later embryonic stages than in earlier stages. This work suggests that differential mRNA decay across cell states and time helps to shape developmental gene expression.

Our ongoing work seeks to elucidate mechanisms behind differential mRNA decay throughout embryogenesis. RNA-binding proteins have gene expression functions that include regulating RNA splicing, localization, translation, and turnover. Determining the regulatory networks that they are involved in will thus be important for a comprehensive understanding of gene regulation during development. We updated the wRBP1.0 compendium of putative *C. elegans* RNA-binding proteins (RBPs) to 928 proteins in WS290 and have catalogued their expression and developmental phenotypes using existing functional genomic resources. Many RBP genes are expressed in a cell type- and developmental stage-specific manner in the embryo, which highlights the potential for RBPs to contribute to distinct gene expression patterns. Furthermore, loss of RBPs can result in a wide range of developmental defects. This updated compendium will provide a resource for functional studies of RBP regulatory networks in *C. elegans*.

54 The role of LIN-41 RNA binding protein in the neuronal maturation of *Caenorhabditis elegans* hermaphrodites

Craig R Peters, Dalton C Patterson, Hee Kyung Lee, Sharrell Greene, HaoSheng Sun Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham

Across the animal kingdom, the number of post-mitotic neurons remains relatively constant from adolescence to adulthood. However, these neurons undergo extensive maturation changes crucial for developing mature behaviors. The regulatory mechanisms that control these maturation changes are not well understood. The evolutionarily conserved RNA-binding protein LIN-41, orthologous to vertebrate TRIM71, is of interest due to its role in regulating the temporal progression of mitotic cell types (e.g., epithelial seam cells) and the maturation of the male nervous system during the *C. elegans* L4 (analogous to puberty) to adult transition. In this study, we investigated the role of LIN-41 in the maturation of the *C. elegans* hermaphrodite nervous system. We characterized LIN-41 expression in the entire nervous system and found a dramatic decrease in expression during the later larval stages (L3/L4) to adult transition, similar to mitotic cells. To investigate its function, we depleted LIN-41 post-embryonically using the auxin-inducible degron (AID) system and found that late larval animals demonstrated adult-like forward/backward locomotion. Focusing on the AIB interneuron, known to control this behavior, we overexpressed *lin-41* specifically in AIB and found that adults exhibited juvenized forward/backward locomotion. To identify downstream genes targeted by LIN-41 that govern these behavioral changes, we analyzed AIB neuronal transcriptome across development and identified the cadherin molecule, *cdh-5,* as a target of interest due to its role in synaptic connectivity. We found CDH-5 nervous system expression to be localized to AIB and developmentally downregulated during the late larval to adult transition, mirroring LIN-41. Additionally, *cdh-5* mutants exhibited precocious behavioral changes as *lin-41* mutants, and we are currently investigating whether these behavioral changes are due to differences in AIB synaptic connectivity. In the future, we plan to utilize single-cell sequencing to examine LIN-41's broader role in neuronal maturation across the entire nervous system. This study aims to enhance our understanding of nervous system maturation during the crucial puberty-to-adult transition and provide insights into how dysregulation of these processes may lead to neurodevelopmental disorders associated with pubertal timing.

55 Single-cell transcriptomic profiling of the Q neuroblast lineage during migration and differentiation

Felipe L. Teixeira[1], Brian J. Sanderson[2], Celeste J. Gormly[1], Erik A. Lundquist[11]Molecular Biosciences, University of Kansas, [2]University of Kansas

Proper migration and differentiation of neuroblasts are crucial for the development of a healthy nervous system. We used a single-cell RNA sequencing (scRNA-seq) approach to study the molecular dynamics of Q neuroblast migration and differentiation at single-cell resolution in *C. elegans.* These bilateral neuroblasts migrate in an asymmetric pattern from similar positions on the left (QL) and right (QR) lateral side of the animal, with QL migrating posteriorly and QR migrating anteriorly. Both neuroblasts divide as they migrate and their descendants continue the asymmetric migration along the anterior-posterior axis, so each lineage will ultimately give rise to three neurons. We isolated Q lineage cells by FACS and used scRNA-seq on the sorted cells, which should include all cells in the Q lineage, to investigate the molecular mechanisms controlling the asymmetrical migration and differentiation of these neuroblasts. Analysis of the scRNA-seq data resulted in a robust transcriptomic differentiation map of the Q neuroblast lineage, which has shown a higher-than-expected similarity between L/R Q neuroblasts during the early stages of Q development. Overall, L/R differences were not as deep as lineage differentiation, and clustering happened more often by Q cell type (i.e., Qx, Qx.a, Qx.p) rather than by L/R asymmetry (i.e., QL.x, QR.x). This is consistent with MAB-5 being a Hox terminal selector on the QL lineage and suggests a robustness in the Q lineage development programming that seems independent of the migration asymmetry. Differential expression (DE) analysis revealed new candidate genes that have not been previously described on the Q cells but that seem to be associated with the development of these neuroblasts. Additionally, DE analysis of previously described genes allowed us to see their expression pattern throughout the Q lineage and to track in which cell types within the Q lineage they are expressed. For example, *sem-2*, a gene downregulated in Q cells in a *mab-5* lof mutant, is expressed mainly in the QL.p lineage. Analysis of Q development in a *sem-2* mutant background showed that defects are, in fact, more common in that lineage. DE analysis also helped us identifying genes that have been associated with Q cell migration but that might act non-autonomously on the Q cells. The low overall expression of *dpy-17* and *sqt-3* throughout the Q lineage, for example, suggests a non-autonomous effect of those genes in their role in early Q cells.

56 Prion-like patterns of inheritance in *C. elegans*

Orkan Ilbay, Thomas Lozanoski, Rebecca Zabinsky, Daniel F Jarosz, Andrew Fire Stanford University

Like DNA, protein molecules can encode information that can be copied, amplified, and transmitted both vertically and laterally. Prion diseases in mammals and yeast prions illustrate the critical importance of information stored and inherited by proteins, which can be either detrimental or adaptive. It remains unknown whether protein-based inheritance can operate more broadly or be transmitted through metazoan germ cells. Here, we report prion-like patterns of inheritance in the model organism *C. elegans*. When genetically fused with a *C. elegans* protein, prion sequences from yeast induce spontaneous gene inactivation, which can then be inherited dominantly, reversibly, and non-chromosomally. The switching between active and inactive states, along with the inheritance patterns observed in *C. elegans*, is reminiscent of prion-like mechanisms in yeast, suggesting that self-perpetuating protein structures may have the capability to serve as effective epigenetic information carriers in *C. elegans*.

57 Giant KASH proteins, nuclear lamins, and ribosomes shape cytoplasmic biophysical properties *in vivo*

Xiangyi Ding[1], Hongyan Hao[2], Daniel Elnatan[3], Patrick Neo Alinaya[2], Ellen Gregory[2], Shilpi Kalra[2], Abby Kaur[2], Sweta Kumari[2], Liam Holt[4], Gant Luxton[2], Daniel Starr[21]Molecular and Cellular Biology, University of California, Davis, [2]University of California, Davis, [3]MCB, University of California, Davis, [4]NYU Grossman School of Medicine

Cytoplasmic biophysical properties govern essential processes from protein folding and phase separation to mechanical stiffness, yet how organisms regulate these properties remains poorly understood. Using genetically encoded multimeric nanoparticles (GEMs) for *in vivo* rheology in *C. elegans*, we discovered uniquely constrained cytoplasmic environments compared to single-cell systems. Our research identifies two key mechanisms regulating mesoscale cytoplasmic properties: ribosome-mediated crowding and structural constraints imposed by the giant KASH protein ANC-1. The transmembrane α-helix of ANC-1 - independent of its canonical nuclear anchoring function - establishes size-dependent constraints on macromolecular diffusion, likely through mechanical scaffolding of the endoplasmic reticulum. Our novel findings implicate specific nuclear envelope proteins in this regulation. Emery-Dreifuss muscular dystrophy (EDMD)-associated LMN-1 variants (R64P and Y59C) cause severe nuclear anchorage defects while affecting GEM mobility similar to ribosome depletion. LMN-1 is required for maintaining endoplasmic reticulum morphology, while other nuclear envelope proteins (UNC-84, UNC-83, EMR-1, LEM-2) do not significantly affect cytoplasmic constraint or crowdedness. These discoveries establish a comprehensive framework for understanding how cells regulate cytoplasmic biophysical properties in living tissues and provide insights into cellular defects in diseases such as EDMD. Our work bridges fundamental cell biology with disease mechanisms through innovative *in vivo*methodologies uniquely available in *C. elegans.*

58 Cell lineage-resolved embryonic morphological map reveals novel signaling associated with cell fate and size asymmetry

Guoye Guan[1,2], Zelin Li[3], Yiming Ma[4], Pohao Ye[4], Jianfeng Cao[3], Ming-Kin Wong[4], Vincy Wing Sze Ho[4], Lu-Yan Chan[4], Hong Yan[3], Chao Tang[2], Zhongying Zhao[41]Harvard Medical School and Dana-Farber Cancer Institute, [2]Peking University, [3]City University of Hong Kong, [4]Hong Kong Baptist University

How cells change shape is essential for the development of organs and embryos. However, studying these changes in detail is challenging. Here we present a comprehensive real-time map that covers over 95% of the cells formed during *Caenorhabditis elegans* embryogenesis, featuring nearly 400,000 3D cell regions. This map includes information on each cell's identity, lineage, fate, shape, volume, surface area, and contact area, along with gene expression profiles, all available through our user-friendly software and website. Our map allows for detailed analysis of important developmental events, such as dorsal intercalation, intestinal formation, and muscle assembly. We also show how Notch and Wnt signaling pathways, along with mechanical forces from cell interactions, influence cell fate and size asymmetries. Our findings suggest that repeated Notch signaling helps create size differences in the large excretory cell, which functions like a kidney. This work sets the stage for in-depth studies of the factors controlling cell fate differentiation and morphogenesis. (In Press in *Nature Communications*)

59 An ancient and essential miRNA family controls cellular interaction pathways in *C. elegans*

Emilio M Santillan[1], Eric D Cormack[1], Jingkui Wang[2], Luisa Cochella[11]Molecular Biology and Genetics, Johns Hopkins University School of Medicine, [2]Institute of Molecular Biology Austria

The transition from unicellular to multicellular life required the acquisition of coordinated and regulated cellular behaviors, including adhesion and migration. In metazoans, this relies on cell adhesion proteins, signaling systems, and an elaborate extracellular matrix (ECM) that contributes to cell adhesion and to the milieu in which signaling interactions occur. Innovations in these pathways that enabled complex multicellularity occurred at the level of new genes and novel functions for existing genes, but also at the regulatory level. Gene regulation by microRNAs expanded with the evolution of multicellularity, yet the functions of individual microRNAs in this context are largely unexplored.

About 30 miRNAs are conserved across bilaterians, but a single miRNA, miR-100, has remained conserved across eumetazoans (animals with true tissues organized into germ layers, including bilaterians and cnidarians). Despite its conservation, the molecular function of miR-100 is practically unknown. In *C. elegans*, miR-51 is an ortholog of miR-100 and it forms a family with six members (miR-51 through -56) that share the seed sequence. Deletion of all six family members causes completely penetrant embryonic defects and lethality, which can be rescued by overexpression of any individual member, indicating that they act in a redundant manner (Alvarez-Saavedra and Horvitz, 2010; Shaw *et al.* 2010). We thus used *C. elegans* to dissect the molecular function of miR-51/miR-100 but also to understand the need for multiple members of this family.

Here, we reveal the molecular function of the homolog of miR-100 in *C. elegans*, the miR-51 family. The miR-51 family acts in a dose dependent manner to control morphogenesis by regulating several genes involved in cell signaling, adhesion and migration, including modifiers of the ECM, specifically heparan sulfate sulfotransferases (HSTs). We provide rigorous genetic validation of functionally relevant targets. Specific HSTs and signaling pathway components are also predicted conserved targets of miR-100 across vertebrates. Our work suggests that this miRNA provided an innovation in the regulation of cellular interactions early in metazoan evolution as animals evolved to form more complex bodies.

60 A 4D transcriptomic atlas for the *Caenorhabditis elegans* embryo

Ryan Christensen[1], Matthew Chaw[2], Richard Ikegami[3], Kaitlyn Boone[3], Alexandra Bokinsky[4], Andrew Lauziere[3], Stephen Xu[3], Brandon Harvey[5,6], Alyssa Stark[3], John Walsh[3], Brie Yarbrough[3], Anthony Santella[7], Mark Moyle[8], Min Guo[9], Yicong Wu[10], Harshad D Vishwasrao[10], Ben Arthur[3], Mark Kittisopikul[3], Daniel Colón-Ramos[11], Zhirong Bao[7], Tosif Ahamed[3], Hari Shroff[11]MTI, Janelia Research Campus / HHMI, [2]University of Washington, [3]Janelia Research Campus / HHMI, [4]CIT, National Institutes of Health, [5]University of California Davis, [6]Emory University, [7]Sloan-Ketterning Institute, [8]Brigham Young University-Idaho, [9]Zhejiang University, [10]National Institutes of Health, [11]Yale University

Understanding how genetic and non-genetic mechanisms combine to orchestrate tissue development is a significant challenge. Due to its invariant cell lineage, limited number of cells, experimental accessibility, and conserved genetics, *Caenorhabditis elegans* is an attractive system for examining how these interactions occur during embryogenesis. To make headway on this topic, our lab has combined 4D positional tracking of nuclei with published scRNA-seq data to generate a 4D transcriptional atlas of the embryo. Our 4D positional atlas provides coverage of all cells from the four-cell stage until movement begins, with an additional 346 nuclei tracked from the beginning of movement until hatching. Mapping publicly available scRNA-seq data onto the cells in our positional atlas allows for the discovery of novel relationships between gene expression and development. For example, we examine the localization of collagens in the developing cuticle, demonstrating that collagen expression patterns in hypodermal cells can predict COL-76 localization to the annular furrow. In a second case study, we investigate how embryonic elongation affects morphology in the developing nerve ring, hypothesizing that elongation is required to establish specific electrical synapses between paired neurons across the ventral side of the nerve ring. To disseminate the use of our resource by the community, we have packaged the 4D transcriptomic atlas as a downloadable software "app" with an interactive viewer to display the expression of individual genes, as well as a clustering function to enable larger-scale analysis of gene expression.

61 Glia-derived serotonin is required for nose touch responses in *C. elegans*

David R Logan[1], Bianca Graziano[2], Lei Wang[1], Melisa L Lamberti[1], Olivia R White[1], Carlos D Oliva[1], Isabella Gay[1], Laura Bianchi[11]Physiology and Biophysics, University of Miami, [2]University of California San Francisco

The sense of touch is essential for our survival and for the care of our young. Touch receptors embedded in the human skin are composed of nerve endings and accessory cells, including glia. Glia and accessory cells are recognized increasingly for their role in the sense of touch. However, the molecular crosstalk between these cells and sensory touch neurons remains unclear. We demonstrate that serotonin produced by glia of *C. elegans* nose touch receptors is important for the regulation of excitability of touch neurons and for behavioral nose touch responses. Using behavioral and genetic approaches, and in vivo Ca2+ imaging, we show that knockdown in glia of *tph-1*, the serotonin synthetic enzyme, and of *cat-1*, the vesicular monoamine transporter, reduces the excitability of touch receptors and the behavioral response to touch. Further, we show that serotonin is likely released via dense core vesicles, as similar phenotypes can be observed in nematodes in which *unc-31*, a vesicular protein needed for fusion of dense core vesicles, is lost in glia. Additionally, we determined that the neuronal G-protein coupled receptor SER-5 is required for neuronal and behavioral responses to touch, suggesting that it is the neuronal receptor that responds to glial serotonin. Excitingly, we also identified a negative feedback loop mediated by the glial serotonin receptor *mod-1* that is important for maintaining normal excitability of both glia and neuron. This work identifies glial serotonin as a complex regulator of touch, and highlights conservation of function for accessory cells in mechanosensation.

62 Ribocin is a nuclease produced by the bacterial pathogen *P. aeruginos* that mediates a novel mode of host translational inhibition during infection of *C. elegans*

Alejandro Vasquez Rifo[1], Denis Susorov[2], Emily H Sholi[2], Jihui Sha[3], James A Wohlschlegel[3], Andrei Korostelev[2], Victor Ambros[21]Molecular Medicine, UMass Medical School, [2]UMass Medical School, [3]University of California, Los Angeles

The interplay between pathogen-associated translation inhibition (TI) and host homeostatic pathways induced by TI critically determines the survival and health outcomes of infections. *Pseudomonas aeruginosa* (*Pae*), an environmental facultative pathogen with a broad host range, induces TI in a range of hosts, including mammals and *C. elegans*. One well-known TI strategy employed by *Pae* against mammalian cells involves the ToxA toxin, which inhibits translation elongation factor 2 (EEF2). Through studies in *C. elegans*, we recently discovered a second *Pae*-induced TI strategy known as 'ribosome cleavage'. Exposure of the nematode to virulent *Pae* strains leads to efficient cleavage of host ribosomes at helix 69 (H69), a highly conserved 26S rRNA hairpin essential for translation.

Using an activity-based fractionation approach, we identified the molecular effector directly responsible for ribosome cleavage — a previously unknown bacterial nuclease that we named ribocin. The *Pae* gene encoding ribocin (*rbcN*), is necessary for *Pae* to induce ribosome cleavage at H69, contributes to bacterial virulence, and fully mediates the induction of the *zip-2/irg-1* pathway under slow killing (SK) assay conditions. Moreover, heterologous *rbcN* expression in *E. coli* is sufficient to induce H69 cleavage and *zip-2/irg-1* pathway activation in worms feeding on the *rbcN* expressing in *E. coli*. Through *in vitro* assays, we established that wild-type recombinant RbcN — but not an H60A mutant — is sufficient to induce H69 cleavage and inhibit translation in rabbit reticulocyte lysates. Moreover, we found that *rbcN* and *toxA* do not act redundantly to affect worm survival to *Pae* or *zip-2/irg-1* pathway activation. Altogether, ribosome cleavage at H69 is mediated by the RbcN nuclease, leading to TI, induction of the *zip-2/irg-1* pathway, and full *Pae* virulence towards *C. elegans*. This second TI strategy predominates over ToxA in the interaction of *Pae* with *C. elegans*.

63 The KASH protein UNC-83 differentially regulates kinesin-1 activity to control developmental stage-specific nuclear migration

Selin Gumusderelioglu[1], Natalie Sahabandu[1], Daniel Elnatan[1], Ellen F. Gregory[1], Kyoko Chiba[2], Shinsuke Niwa[2], G. W. Gant Luxton[1], Richard J. McKenney[1], Daniel A. Starr[1]1Department of Molecular and Cellular Biology, University of California Davis, [2]Frontier Research Institute for Interdisciplinary Sciences, Tohoku University

Nuclear migration plays a fundamental role in development, requiring precise spatiotemporal control of bidirectional movement through microtubule motor proteins. The *C. elegans* KASH protein UNC-83 is a nuclear-envelope cargo adaptor that exists in multiple isoforms and directly interacts with kinesin-1 and cytoplasmic dynein motors. Kinesin-1 is the main motor driving embryonic hyp7 precursor nuclear migration while dynein is predominant during larval P-cell nuclear migration. The mechanisms by which UNC-83 regulates these distinct motors to produce opposite directional nuclear movements at different developmental stages is unknown. We hypothesize that nuclear movement directionality in *C. elegans* is determined by the developmentally regulated expression of alternative isoforms of UNC-83. To test this, we used genetic and biochemical methods. We demonstrated that deletion of the N-terminal domain specific to the longer UNC-83a/b isoforms disrupted larval P-cell nuclear migration while not affecting the embryonic hyp7 nuclear migration. We also showed that expression of long isoform *unc-83a* under the promoter of the short *unc-83c* isoform blocked nuclear migration only in embryonic hyp7 precursors. We concluded that the shorter UNC-83c isoform promotes kinesin-1-dependent nuclear movement in embryonic hyp7 precursors, while longer UNC-83a/b isoforms facilitate dynein-mediated nuclear migration in larval P cells. Furthermore, we showed that the N-terminus of UNC-83a is sufficient to inhibit kinesin-1 activity *in vitro*. UNC-83a had a ten-fold weaker affinity for the kinesin light chain (KLC-2) than UNC-83c had, while the N-terminus of UNC-83a bound to the kinesin-1 heavy chain (UNC-116). Thus, we concluded that UNC-83a's N-terminal domain functions as a kinesin-1 inhibitory module by directly binding UNC-116. This isoform-specific inhibition, combined with differential affinity for KLC-2, establishes a molecular switch for directional control. Together, these interdisciplinary studies reveal how alternative isoforms of cargo adaptors can generate developmental stage-specific regulation of motor activity during development.

64 Intestinal RICT-1 regulates the larval germline progenitor pool via the vitellogenin VIT-3 in *C. elegans*

Anke Kloock, E. Jane Albert Hubbard Department of Cell Biology, NYU Grossman School of Medicine

Populations of proliferating cells such as stem cells and tumors are often nutrient responsive. Highly conserved signaling pathways communicate information about the surrounding environmental, organismal, and cellular nutrient conditions. One such pathway is the Target of Rapamycin (TOR) pathway. The TOR kinase exists in two complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2). TORC1 has been researched extensively and its regulation, particularly by amino acids, is well characterized. TORC1 activity promotes both stem cell fate and proliferation in the *Caenorhabditis elegans* hermaphrodite germline stem cell system to facilitate expansion of the larval germline Progenitor Zone (PZ) pool in response to nutrients. By contrast, a role for TORC2 in germline development has not been investigated. Here, we show that RICT-1, the sole *C. elegans* ortholog of the TORC2-specific component RICTOR, also promotes expansion of the larval PZ, acting largely through SGK-1 and SINH-1. Further, unlike the germline-autonomous role for TORC1 components, intestinal *rict-1* is both necessary and sufficient for normal germline PZ pool establishment. Furthermore, neither loss of *daf-16* nor *daf-3* suppresses the *rict-1* PZ pool defect, suggesting that neither the IIS nor DAF-7/TGFß pathway mediates the effects of RICT-1. Rather, intestinal RICT-1 likely acts via vitellogenins, intestinally produced yolk proteins characterized for provisioning the adult germ line, but not previously characterized for a role in larval germ line development. By comparative RNA-seq on staged L4 larvae, we found vitellogenin genes among highly differentially abundant mRNAs. Genetic analysis supports a role for *vit-3* in germline development in a linear pathway with *rict-1*. Our results establish the *C. elegans* germ line as a fruitful model for investigating TORC2 and its connection to stem cells and lipid biology.

65 The NUC-1 Exonuclease acts in the lysosomes of the engulfing cell to degrade apoptotic cell DNA

Jonathan Pickett[1], Niccole Auld[2], Lathan Lucas[2], Omar Peña-Ramos[2], Xianghua Liu[2], Zheng Zhou[2]1Biochemistry and Molecular Pharmacology, Baylor College of Medicine, [2]Baylor College of Medicine

Approximately 300 billion cells die and need to be replaced in our bodies every day. Degrading such vast amounts of cellular content, in addition to foreign material such as that of bacteria, requires the coordinated activity of specialized enzymes and subcellular compartments. One of the most important enzymes in this process, deoxyribonuclease-2 (DNAse-2), is also responsible for preventing unnecessary immune responses to DNA. *nuc-1* is the *Caenorhabditis* gene that encodes the DNAse-2 enzyme. NUC-1 is primarily localized to lysosomes where the acidic environment allows this enzyme to function at its peak efficiency. However, some reports propose that NUC-1 may act in the nucleus of apoptotic cells to degrade chromosomal DNA after it is fragmented. Whether NUC-1's function is required in the engulfing or apoptotic cells for apoptotic cell DNA degradation has not been unambiguously determined. To clarify this important issue, we have developed time-lapse imaging assays to monitor the degradation of apoptotic cell DNA in *C. elegans* embryos. Contrary to the previous reports, we show that NUC-1 acts in the engulfing cells and inside phagolysosomes to degrade apoptotic cell DNA. Subcellular localization study of NUC-1 in both the engulfing and apoptotic cells supports the DNA degradation role of NUC-1 inside the phagosomal lumen. We conclude that the engulfment of apoptotic cells and lysosomal fusion to phagosomes are required for NUC-1-mediated apoptotic cell DNA degradation. In the future, we will identify additional enzymes responsible for the degradation of specific categories of molecules of apoptotic cells.

66 A role for the spectrin cytoskeleton in cuticle alae patterning

Prioty Sarwar[1], Meera Sundaram[21]Genetics, University of Pennsylvania, [2]University of Pennsylvania

The apical extracellular matrix (aECM) lines, shapes and protects exposed surfaces in animals and often organizes to form elaborate three-dimensional structures like the scales of a butterfly wing or *Drosophila* denticles. How animals pattern these large structures during development is poorly understood. The *C. elegans* alae, cuticle ridges that run along the sides of the worm in three long 'racing' stripes, are an example of one such extracellular structure. We are studying how the cytoskeleton patterns *de novo* formation of the adult alae.

Previous research found that four longitudinal actin filament bundles (AFBs) form at the lateral (seam) epidermis and pattern assembly of a transient pre-cuticle aECM that is required for alae formation. Additionally, ultrastructural data revealed a potential role of pre-cuticle delamination in alae morphogenesis, where four small horizontal areas of delamination demarcate the valleys between alae, while remaining points of matrix adhesion demarcate the developing alae ridges. One hypothesis generated by this observation is that the longitudinal AFBs exert mechanical forces on the overlying matrix to trigger pre-cuticle delamination.

We tested several cytoskeletal proteins that have actin binding and plasma membrane binding domains, making them ideal candidates to aid in the assembly of seam AFBs and/or facilitate AFB-mediated mechanical forces on the matrix. Super-resolution imaging shows that the spectraplakin VAB-10 and intermediate filament bundles are arranged in a single line at the middle of the seam epidermis offset from AFBs, while the apical bH-spectrin SMA-1 is found superimposed on the AFBs. *sma-1* loss specifically disrupts organization of the two medial-most seam AFBs that would normally flank the site where the central ridge forms. Correspondingly, *sma-1* loss also disrupts continuity of the pre-cuticle and cuticle matrix specifically in this central ridge region. *sma-1* mutants appear to have increased cortical actin tension within the seam, as suggested by increased localization of a mechanosensitive LIM-domain protein. We hypothesize that the combination of increased and dispersed cortical actin tension could lead to broader domains of matrix delamination that prevent the central alae ridge from forming; ultrastructural studies are underway to test this prediction. Overall, our data support the hypothesis that mechanical forces relayed by cytoskeletal structures play a role in the formation of the *C. elegans* adult alae.

67 Differential contributions of b-tubulin isotypes maintain a balance between microtubule-crosslinking and microtubule-severing activities in *C. elegans* oocyte spindles

Emmanuel Nsamba, Anne Villeneuve Developmental Biology, Stanford University

Assembly of bipolar spindles without centrosomes, a universal feature of oocyte meiosis, involves coordination between microtubules (MTs) composed of several different α- and β-tubulin isotypes, MT associated motors and other MT-interacting proteins (MAPs). Here we use high-resolution immunofluorescence and live imaging and *Caenorhabditis elegans* strains with altered β-tubulin isotype composition to investigate the contributions of and interplay between MT composition, motors, and MAPs during meiotic spindle assembly and function.We found that "TBB-2 only" oocytes (containing only one of two partially-redundant germline-expressed β-tubulin isotypes) exhibit a synthetic genetic interaction with *klp-18(ts)*, a missense mutation causing partial loss-of-function of the plus-end directed kinesin-12 MT cross-linking motor KLP-18, resulting in a high incidence of embryo lethality, chromosome missegregation and monopolar oocyte spindles. Moreover, these synthetic spindle assembly and chromosome segregation defects can be suppressed either by a *tbb-2(E439K)* mutation that confers partial resistance of MTs to the MT-severing enzyme katanin or by a *mei-2(A235T)* mutation that decreases katanin activity. We further found that both spindle length and the spatial distribution of katanin along spindles are affected by β-tubulin isotype composition, with "TBB-1 only" oocytes exhibiting longer meiotic spindles and a dearth of katanin at spindle centers relative to poles. We are also using live imaging of oocyte meiosis to investigate potential impacts of tubulin isotype composition on temporal dynamics of spindle assembly and function. Together our data support a model in which *C. elegans* oocyte spindle bipolarity requires a balance between microtubule crosslinking and severing activities, with β-tubulin isotype composition helping to maintain this balance.

68 AltZyxin, an alternative protein translated from the same transcript as Zyxin, regulates muscle biology

Noémie Frébault[1,2], Marin Pascal[1,2], Ivan Valette[1,2], Lise Rivollet[1,2,2], Benoît Grondin[1,2], Tianyou Wang[1,2], Thomas Boulin[3], Benoît Vanderperre[1,2], Claire Bénard[1,2,41]Dept. Biological Sciences, Université du Québec à Montréal, [2]CER-MO-FC Research Center, [3]MeLiS, Université Claude Bernard Lyon 1, [4]Dept Neurobiology, University of Massachusetts Chan Medical School

The recent discovery of “alternative proteins”, which are encoded on the same transcript as “canonical proteins” but on different reading frames, adds ~30 000 novel proteins to the human proteome significantly expanding the landscape of biological functions. Yet, alternative proteins remain largely unexplored. Among the few alternative proteins studied so far, some physically interact with the canonical protein encoded by the same gene, modulating their expression, localization, and function. Other alternative proteins can function independently of the canonical protein. One example of an alternative protein is AltZyxin/ALTZYX-1, which is encoded by the conserved gene *ZYX/zyx-1* that also encodes the canonical protein Zyxin/ZYX-1. AltZyxin is an abundant and conserved alternative protein, produced from an alternative ORF in the 5›UTR –as revealed by mass spectrometry studies in both C. elegans and humans. AltZyxin functionality and cell biological roles is poorly understood. Previous studies showed that the gene *zyx-1* is required for synapse maintenance and muscle health, as either overexpression or loss of *zyx-1* alleviates dystrophin-dependent muscle degeneration. Here, we investigate the function of AltZyxin, including in synapse and muscle biology, which is key to decipher the mechanism by which the gene *zyx-1* regulates synaptic and muscle maintenance. For this, we generated a series of CRISPR-engineered mutations to specifically disrupt either AltZyxin or Zyxin, and study the phenotypic consequences resulting from their single or combined loss. AltZyxin loss does not impact neurons nor synapses, but profoundly affects muscle cells, including their mitochondria and sarcomere organization, which is consistent with it being expressed specifically in muscles, as we have found. Interestingly, AltZyxin localizes to both nuclei and sarcomeres, where we show that it colocalizes with myosin. Our multiple analyses of muscle phenotypes, body length, and lifespan, among others, uncover a functional interplay between Zyxin and AltZyxin, where the loss of AltZyxin antagonizes the loss of Zyxin. To get further insights into the function of AltZyxin in muscle cells, we are carrying out an in vivo TurboID screen to identify AltZyxin potential interactors. In sum, this work reveals a critical role for an alternative protein in muscle biology, which modulates Zyxin›s role, and underscores the importance of considering alternative proteins in cell biology and diseases.

69 A doublecortin-like kinase regulates the development of mechanosensory cilia

Adria Razzauti Sanfeliu Genetics, Boston Children’s Hospital - Harvard Medical School

Doublecortin (DCX) and Doublecortin-like kinases (DCLK) are essential for proper neuronal migration and cortical layering during brain development. Both DCX and DCLK contain N-terminal Doublecortin (DC) domains that serve as tubulin-binding sites to promote microtubule polymerization and support axon and dendrite outgrowth in developing neurons. In contrast to DCX, DCLK also contains a C-terminal kinase domain. While much focus has been placed on the role of the DC domains, the kinase activity of DCLK has received less attention. Here, we find that the mutant *cat-6(e1861),* which was isolated more than 40 years ago, disrupts *F32D8.1*, a *C. elegans* ortholog of human DCLK3 that shares 40% identity in the kinase domain but lacks the DC domains. In classical work, *cat-6* was found to display ultrastructural defects in the mechanosensory CEP cilia, as well as an abnormal opening in the overlying cuticle that leads to inappropriate staining of CEP cilia with the lipophilic dye FITC (Perkins et al., 1986). We found that the *cat-6(e1861)* mutant contains a nonsense mutation in *F32D8.1* and that both an existing partial deletion (*tm4001)* and a complete deletion we generated *(hmn444)* recapitulate the FITC staining phenotype, suggesting that *F32D8.1* is *cat-6.* A transcriptional reporter shows *cat-6* is expressed in ciliated mechanosensory neurons (CEP, OLQ, OLL, and IL1). We found that mutations in *cat-6* lead to severely disrupted CEP and OLQ cilia morphology, including distal cilia truncations and misshapen endings. We find that both CEP and OLQ stain with FITC in the mutant. Rescue experiments indicate that *cat-6* acts cell-autonomously to regulate both cilia shape and FITC staining. Examination of an endogenous fluorescent tag showed that CAT-6 localizes almost exclusively to CEP, OLQ, OLL and IL1 cilia. Surprisingly, its expression is regulated during the molt cycle, turning on during molts and remaining undetectable during intermolt or adult stages. Its regulated temporal expression, along with our lab’s observations that mechanosensory cilia undergo shedding and regrowth during each molt cycle (Swope and Heiman, unpublished), suggests that the CAT-6 kinase potentially acts in cilia regrowth after molting. Together, our results resolve a >40-year-old mystery and suggest that, in addition to established roles for DCX and DCLK proteins in axon and dendrite microtubule dynamics, a DCLK kinase may also play critical roles in cilia morphogenesis.

70 Elucidating the trafficking mechanism of electrical synapse components

Sudhanand Muraleedharan, Atal Vats, Abhishek Bhattacharya National Centre for Biological Sciences

While chemical synapses have been widely studied, another major mode of neuronal communication, the electrical synapses, remain much under-explored, despite their conserved roles in neural circuit development and function. Electrical synapses are specialized trans-cellular connections formed by transmembrane connexin proteins in vertebrates and innexins in invertebrates, which enable direct ionic communication between neurons. These synapses are composed of clustered gap junction channels at precise neuronal locations. An essential first step to understand the cell biological principles contributing to the synaptic partner choice, development and plasticity of these synapses is to understand the trafficking mechanisms of electrical synapse components at synapse level resolution; areas that are rarely studied.

Using genetic screens in *C. elegans*, we uncovered that neurons employ distinct trafficking mechanisms to target molecularly diverse gap junction channels within individual "heterochannel" synapses. For the first time, we identified that channels formed by the innexin, INX-1, is transported by KLP-4, a conserved kinesin-3 family of anterograde motor proteins (homologous to KIF13A/B and KIF14), and a kinesin-14 family member KLP-3 (homologous to KIFC2/3), in a neuron-specific manner. Intriguingly, trafficking of two other innexins, UNC-7 and UNC-9, which co-localize at the same synapse, depend on KLP-3, but is independent of KLP-4. These suggest a novel mechanism where distinct trafficking pathways regulate the localization of multiple innexins to a single synapse. Furthermore, during the metabolically distinct dauer diapause stage, we observed spatiotemporal regulation of kinesin-motor expression, leading to neuron-specific alterations in kinesin dependency for innexin transport. This ultimately leads to, a) stage-specific utilisation of distinct kinesins and b) plasticity of electrical synapses with respect to their innexin composition.

From an ongoing genetic screen, we have further identified multiple candidate adaptor/accessory proteins that regulates INX-1 trafficking. By further expanding this list of accessory proteins and identifying specific protein domains responsible for specialized innexin trafficking, we aim to dissect the mechanisms underlying electrical synapse formation and plasticity. Overall, our study advances understanding of gap junction biology and provides a foundation for exploring their roles in neural function and connectivity.

71 RanGAP prevents premature centriole disengagement in the *C. elegans* germline

Laura L Thomas[1], Ana L Curinha[2], Geraldine Seydoux[21]HHMI / Johns Hopkins University School of Medicine, [2]Molecular Biology and Genetics, HHMI / Johns Hopkins University School of Medicine

Centrosomes are evolutionarily conserved organelles that play a key role as the major microtubule organizing center. Centrosomes consist of a pair of centrioles surrounded by the pericentriolar material (PCM). Centrioles are duplicated during each cell cycle and each centriole pair remains attached before disengaging during late mitosis. Prior studies have described roles for Polo-like kinase 1 (PLK-1) and Separase (SEP-1) in facilitating centriole disengagement. However, the mechanisms controlling centriole separation and eventual elimination in oocytes remain poorly understood.

Here, we find a surprising novel role for the nuclear envelope associated protein RanGAP (RAN-2) in controlling centriole disengagement in the *C. elegans* germline. We find that endogenously tagged RanGAP localizes dynamically to centrioles: RanGAP is absent from unpaired centrioles in the mitotic zone but localizes to paired centrioles in the pachytene region. RanGAP enriches in a distinct layer surrounding the PCM component SPD-2, suggesting that RanGAP is a component of an outer layer of the PCM. Strikingly, acute RanGAP depletion using the auxin-inducible degron system causes highly penetrant premature centriole disengagement in the pachytene germline.

To our knowledge, this is the first reported example of a nuclear envelope protein moonlighting to control centrosome dynamics. We will describe our ongoing work using genetics and expansion microscopy to understand how RanGAP prevents premature centriole disengagement in the *C. elegans* germline.

72 Sexual dimorphisms in meiotic chromosome structures drive heat induced male infertility in *C. elegans*

Cori Cahoon[1], Nicolas Lee[2], Karissa Coleman[1], Cailan Feingold[3], Diana Libuda[31]Colorado State University, [2]Fred Hutchinson Cancer Center, [3]University of Oregon

Many developmental processes critical for the genome stability of developing sperm and egg are directly altered by environmental stressors, such as temperature, toxins, and pH. In contrast to egg development, developing sperm are incredibly sensitive to changes in temperature with heat exposure strongly linked to genome instability, infertility, and cancer. Despite these detrimental consequences of heat on male reproductive health, the mechanisms behind heat-induced male infertility are largely unknown. Here we show in the nematode *Caenorhabditis elegans* that differences in the temperature sensitivity between sperm and egg development may be due to sexual dimorphisms in the synaptonemal complex (SC), a conserved, meiosis-specific chromosome structure required for meiotic genome stability and fertility. We find that only the spermatocyte SC is destabilized and fragmented following acute heat exposure. Concurrently, heat exposure also causes an increase in double strand DNA breaks. To establish the sexually dimorphic features rendering the SC temperature sensitive, we developed novel imaging tools for sex comparative live imaging studies in *C. elegans* and identified several sexual dimorphisms in SC composition and dynamics without heat exposure. We also uncover functional roles for these sex-specific differences in SC composition where individual SC central region proteins regulate specific steps of meiotic DNA repair differently in each sex. Finally, we performed a forward genetic screen and identified mutants that both suppress and enhance the sex-specific mechanisms regulating heat sensitivity of the SC, including a suppressor mutation in the conserved FERM-domain containing protein FRM-1. Together, these studies reveal the sexual dimorphisms within the SC and provide insights into how developing sperm and eggs adapted similar chromosome structures to differentially regulate and execute meiotic stress responses to temperature.

73 The role of DNA topology in meiosis and genome integrity

Rui Jiang[1], Hyung Jun Kim[1], Cyrus Ruediger[1], Peter Meister[2], Elçin Ünal[1], Monica Guo[3], Abby Dernburg[11]University of California, Berkeley, [2]University of Bern, [3]University of Washington

Meiosis is essential for sexual reproduction. Recombination during meiosis mixes information between two parental genomes and ensures accurate chromosome segregation. Meiotic recombination begins with programmed DNA double-strand breaks (DSBs) catalyzed by Spo11/SPO-11, which preferentially occur in genomic regions known as "hotspots." In most vertebrates, hotspots are specified by PRDM9, whereas in invertebrates, DSBs concentrate near promoters. This is likely due in part to chromatin accessibility, but the genomic features that promote DSBs remain elusive. Since Spo11 is related to topoisomerases, we hypothesize that it may preferentially cleave torsionally stressed DNA.

To explore the roles of DNA structure in DSB formation and other genome functions, we are developing genome-wide methods to profile DNA structure in *C. elegans* germline. While high-resolution cytological analysis using *C. elegans* germline has been instrumental in studying meiotic chromosome dynamics, genome-wide chromatin profiling has been limited by the need for large-scale isolation of germ nuclei, requiring ~1 million worms per ChIP-seq experiment. This constraint makes it impractical to use ChIP-seq to analyze mutants that are difficult to grow in bulk. To overcome this challenge, we adapted CUT&RUN and CUT&Tag to profile chromatin in microdissected germlines. We now routinely obtain high signal-to-noise data starting with as few as 15 dissected gonads for abundant histone modifications and 40 gonads for proteins of lower abundance.

Using CUT&RUN and CUT&Tag, we have mapped the genome-wide supercoiling landscape in *C. elegans* germlines. Negative supercoiling was probed by the preferential intercalation of psoralen, while positive supercoiling was detected by expression and binding of GapR, a *Caulobacter* protein that preferentially binds overtwisted DNA. Both forms of supercoiling are enriched at transcription start (TSS) and end sites (TES) of active genes. Intriguingly, meiotic cohesins and DSB-promoting factors also localize to these supercoiled regions. We are now developing strategies to map DSBs and recombination intermediates to explore how DNA structure influences DSB formation and processing.

These methods will greatly lower the technical barriers to chromatin profiling experiments for *C. elegans* researchers.

74 Clathrin-mediated endocytosis associates with transient clusters of branched F-actin in the activated *C. elegans* oocyte

Archit Bhatnagar[1], Subhadip Chakraborty[2], Lily Zahwa Khamizan[3], Deborah Sissay[3], Mohammed Faheem[3], Arjun Narayanan[3], Teije Middelkoop[21]Institute of Biotechnology of Czech Academy of Sciences, Prague, [2]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, [3]New York University, Abu Dhabi

A key event in the embryonic development of *C. elegans* is the activation of a contractile actomyosin cortex during maturation of the oocyte before its fertilization. Recent studies have reported that this cortex activation is supported by the emergence of highly dynamic F-actin clusters on the nascent oocyte cortex. These are primarily composed of WSP-1/N-WASP, the Arp2/3 complex and branched actin filaments, and exhibit stereotypical compositional dynamics. However, the physiological role and function of these dynamic clusters remains unclear. Using high resolution time-lapse microscopy of isolated oocytes we find that the cortical actin clusters co-localize with various components involved in Clathrin-Mediated Endocytosis (CME). Furthermore, blocking CME by either genetic or chemical perturbations affects the distribution, morphology and compositional dynamics of the branched actin clusters. Altogether, our results suggest that the dynamic branched actin clusters are sites of clathrin-mediated endocytosis, and put forward a possible mechanism of how endocytosis can control the activation and remodeling of the actomyosin cortex.

75 Deciphering the mechanisms regulating PIEZO channel membrane trafficking.

Xiaofei Bai[1], Milagros Rincon Paz[2], Yooseong Wang[11]University of Florida, [2]Biology, University of Florida

PIEZO proteins are mechanotransductive ion channels that translate mechanical stimuli into cellular signaling, which are essential for almost all physiological processes. Over 100 pathogenic variants of PIEZO have been associated with at least 26 diseases and disorders, including congenital lymphatic dysplasia, distal arthrogryposis, and perinatal respiratory distress. Despite their clinical relevance, effective therapeutic treatments for PIEZO-associated pathologies remain largely elusive. A limitation to developing targeted treatments is the incomplete understanding of the cellular and molecular natures of the PIEZO channel, including the mechanism regulating PIEZO channel trafficking and localization.

In this study, we leverage the *C. elegans* germline to investigate the cellular mechanisms governing PIEZO protein localization and membrane trafficking. Utilizing a high-resolution confocal imaging system, we characterized the trafficking dynamics of PEZO-1, the C. elegans PIEZO ortholog, endogenously tagged with multiple fluorescent markers. Our findings reveal that GFP::PEZO-1 colocalized with key membrane vesicle markers, including caveolin-1 GFP::CAV-1 and the recycling endosome regulator GFP::RAB-11, underscoring a conserved vesicular trafficking pathway of PEZO-1. Notably, knock-down *rab-11* and a t-SNARE *syx-4*, disturbed GFP::PEZO-1 vesicle transportation to the plasma membrane, leading to its aberrant accumulation in the perinuclear region. These data suggested that PEZO-1 trafficking is dependent on RAB-11-dependent finely tuned vesicular transport machinery.

Furthermore, we demonstrated that the structural integrity of PEZO-1 is required for its proper trafficking. Depletion of either the C-terminal ion pore or N-terminal transmembrane domains, as well as the gain-of-function PIEZO missense mutation R2405P, resulted in severe trafficking defects, vesicular mislocation, and impaired plasma membrane expression. Intriguingly, we uncover a regulatory role of sperm-derived signaling in PEZO-1 trafficking, wherein the absence of sperm, and its major signaling molecule, major sperm protein (MSP), abrogated PEZO-1 cortical enrichment, linking PIEZO channel dynamics to reproductive signaling and oocyte maturation.

Collectively, our findings establish a fundamental cellular framework governing PIEZO protein trafficking and membrane localization. By identifying key vesicular trafficking regulators and structural elements of PEZO-1, our study demonstrates mechanistic insights into the regulation of PIEZO channel membrane dynamics. These findings may inform the development of therapeutic strategies for the medical conditions associated with PIEZO channel dysfunction.

76 Effects of early life adversity on the adult brain of *Caenorhabditis elegans*

Giulio Valperga, Zion Walker, Oliver Hobert Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

Adverse conditions during early life can significantly alter the behaviour, metabolism, and physiology of adult individuals. In the nervous system, early-life adversity causes lifelong gene expression changes through a poorly understood process of biological embedding. In response to aversive conditions, the nematode *C. elegans* enters a developmental arrest stage called “dauer,” which it exits upon sensing more favourable conditions. This transition induces physiological, behavioural, and gene expression changes in adult post-dauer individuals. Here, we characterised behavioural programs altered in post-dauer animals as well as gene expression changes. Passage through the dauer stage persistently increased the priority for food of post-dauer individuals, as highlighted by reduced locomotory activity on food, a sexually dimorphic trait, comparable egg-laying rates ON and OFF food, and elevated feeding. Fasting and starvation caused post-dauer individuals to lower their speed when encountering food, a feature shared with controls, and increase their pumping rate, a feature specific for post-dauer individuals. Using an extensive collection of CRISPR/Cas9-edited, endogenous reporter alleles, we identified robust gene expression changes in specific neurons for many neuropeptide genes, several in both sexes. *flp-10* showed the largest up-regulation in the BAG sensory neurons. Loss of *flp-10* or *egl-6,* its receptor, suppressed the egg-laying phenotype of post-dauer individuals. Among enzymes for classical monoamines, *tph-1*, the serotonin synthesis rate-limiting enzyme, showed the largest change. Loss of *tph-1* affected the speed of post-dauer animals, but not controls, after fast and starvation, suggesting they rely more on serotonin signalling during further starvation. Neuronal gene expression changes in dauers require the nuclear hormone receptors DAF-12/VDR in the nervous system. Using the AID2 system, continuous removal of DAF-12/VDR from the nervous system affected post-dauer gene expression changes. We observed both suppression and enhancement, suggesting DAF-12/VDR might be required to instruct the expression changes of post-dauer adults. Taken together, these data indicate post-dauer individuals utilise neuronal DAF-12/VDR to establish and/or maintain persistent gene expression changes in post-dauer adult individuals, thus likely resulting in altered behavioural programs.

77 Stage-specific circuit rewiring in *C. elegans* dauer preserves avoidance index but shortens reversal response

Daniel T. Choe[1], Junhwan Alexander Bae[2], Myung-kyu Choi[2], Junho Lee[1,21]Seoul National University, [2]The Research Institute of Basic Sciences

During the developmental stages of *C. elegans*, the dauer stage represents an alternative state entered under environmental stress, characterized by distinct behaviors absent in reproductive stages. To investigate the mechanisms underlying these stage-specific behaviors, we reconstructed the dauer nervous system and identified extensive rewiring of downstream signals from sensory neurons in a stage-dependent manner. In this study, we focused on the well-characterized nociceptive response in *C. elegans*. Behavioral assays, optogenetic stimulation, and computational simulations revealed that the avoidance index in the dauer stage remained comparable to that of adult hermaphrodites, despite extensive rewiring of the nociceptive circuitry. Simulations of modified dauer networks confirmed that connectivity between ASH and AVA neurons was the sole critical factor determining the overall avoidance index. In contrast, we found that the reversal wave was significantly reduced in the dauer stage, as demonstrated by both behavioral assays and optogenetic stimulation. We hypothesized that increased gap junctions within the dauer nociceptive circuit contribute to this reduction by facilitating faster signal processing, thereby shortening the overall avoidance response. Supporting this hypothesis, adults engineered to express ectopic gap junctions within the nociceptive circuit exhibited a reduction in the reversal wave similar to that observed in dauers. Calcium imaging of individual neurons in the nociceptive circuit further revealed differences in their responses to sensory cues, concordant with our hypothesis. In summary, despite extensive circuit rewiring, dauers retain their initial avoidance response by preserving core connectivity. However, the duration of the avoidance response is reduced due to increased gap junctions within the network.

78 NCAM (Neural Cell Adhesion Molecule) promotes synaptic remodeling in developing GABAergic neurons

Casey Gailey[1,2], Robert Held[3], Claudia Palacios[3], Andrea Cuentas-Condori[3], Leah Flautt[3], Siqi Chen[3], John Tipps[3], Eleanor Rodgers[4], Seth Taylor[3], Engin Ozkan[5], David Miller[1,2,61]Cell and Developmental Biology, Vanderbilt University, [2]Program in Developmental Biology, Vanderbilt University, [3]Vanderbilt University, [4]Saint Cecilia, [5]University of Chicago, [6]Program in Neuroscience, Vanderbilt University

Neural circuits are actively remodeled during development as new synapses are assembled and others are removed. To investigate the underlying mechanism of synaptic remodeling, we are exploiting the DD-type GABAergic motor neurons. In young larvae, DD presynaptic boutons are initially positioned on ventral muscles but are then relocated to innervate dorsal muscles. We have shown that the transcription factor, IRX-1/Iroquois, activates expression of the sodium epithelial channel (ENaC), UNC-8, to trigger a Ca2+-dependent endocytic mechanism that promotes presynaptic disassembly and recycling to dorsal synapses1.

To identify additional effectors of synaptic remodeling, we used single cell RNA-Seq to profile early larval stage DD neurons. A mutant of one of these genes, *ncam-1,* impairs removal of ventral GFP::RAB-3 and its reassembly at dorsal synapses. NCAM-1 localizes to DD synapses and is also required for normal remodeling of active zone proteins CLA-1 and ELKS-1, which are not affected by *unc-8*. Moreover, genetic epistasis experiments with GFP::RAB-3 suggest that NCAM-1 and UNC-8 function in parallel pathways. NCAM-1 is a conserved member of the Neural Cell Adhesion Molecule (NCAM) family with an extracellular domain composed of Ig and fibronectin repeats. We used CRISPR mutants to show that N-terminal Ig1 and Ig2 domains are dispensable for DD remodeling whereas the NCAM-1 intracellular domain (ICD) is required. These findings suggest that NCAM-1 homophilic interactions mediated by Ig1/Ig2 are not necessary for remodeling but that downstream signaling by the NCAM-1 ICD domain is important. Independent biochemical assays detected strong interaction of the extracellular domains of NCAM-1 with RIG-3, an Ig-domain cell adhesion protein with topological similarity to Drosophila Klingon. Genetic analysis shows that NCAM-1 and RIG-3 function in a common pathway, suggesting that the NCAM-1-RIG-3 complex may mediate synaptic remodeling. Because NCAM can regulate plasticity in mammalian neurons, we suggest that NCAM-1 may drive synaptic remodeling in *C. elegans* in a mechanism that also governs circuit refinement in the developing brain. NIH Funding: T32HD007502, F31NS134292, R01NS10695.

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79 The histone methyltransferase *set-4* regulates expression of *unc-44* and *gap-2* and modifies GABA neuron remodeling

Kristi P. Zoga, Sophia Villiere, Vina Tikiyani, Andrea F Edwards-Cintron, Michael P Hart Genetics, University of Pennsylvania

Variation in more than 100 genes is associated with increased risk for autism, but we do not understand how these genes function to affect overlapping and distinct behavioral changes. Recent efforts have observed phenotypic convergence for autism-associated genes in early steps of neurodevelopment, but with limited mechanistic understanding into this convergence. Further, many autism-associated genes likely also have roles in later maturation, function, and plasticity of circuits. We screened twenty conserved autism-associated genes for impact on DVB GABAergic neuron remodeling, circuit plasticity, and behavior in adult male *C. elegans*. *unc-44/ANK2*, *gap-2/SYNGAP1, set-4/KMT5B, daf-18/PTEN,* and *chd-1/CHD8* function to repress, while *CACNA2D3/unc-36* functions to promote, DVB neurite outgrowth in adult males. We find that expression of the long isoform of *unc-44* increases in adult male tail neurons/muscles and loss of the H4K20 histone methyltransferase *set-4* represses this increasing expression. *gap-2* displays neuron specific dynamic patterns of expression, including a decrease in DVB during early adulthood. Loss of *set-4* results in an earlier decrease in *gap-2* expression in DVB (and other specific neurons). We find little overlap in the genes that impact DVB neurite outgrowth and the corresponding spicule protraction behavior, but epistatic analysis of our candidate genes and *nrx-1/NRXN1* uncovered additive, suppressive, antagonistic, synergistic, and reciprocal sign genetic interactions in neurite outgrowth and behavioral phenotypes. We discovered a novel role for *set-4* in the temporal expression patterns of autism-associated synaptic genes in an isoform- and neuron-specific manner. We also defined gene networks, interactions, and new molecular mechanisms that control GABAergic neuron and circuit remodeling, linking this neuronal process to conserved autism genes.

80 The metalloprotease SUP-17/ADAM10 inhibits axonal repair

Xue Yan Ho[1,2], Keerthana Ponniah[1], Igor Bonacossa Pereira[1,3], Sean Coakley[4], Victor Anggono[1], Massimo A Hilliard-[1]1Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, [2]Department of Biology, Stanford University, [3]Institute for Molecular Biosciences, The University of Queensland, [4]School of Biomedical Sciences, The University of Queensland

Axonal damage, such as in nerve injuries, interrupts the communication between a neuron and its target tissue. Functional recovery is achieved when the regenerating axon reinnervates its original target tissue. However, intervention to repair such damage is still not achievable. Axonal fusion is an efficient means of repair following axonal transection, whereby the proximal axon, still attached to the cell body, regrows and re-establishes membrane and cytoplasmic continuity with its own separated axonal fragment, restoring neuronal function. The molecular mechanisms of this process are not fully elucidated. In *C. elegans*, a key function has been established for the fusogen EFF-1, which mediates the merging of the plasma membranes of the two separated fragments. Using a candidate gene approach, and the *C. elegans* PLM mechanosensory neurons as a model system, we identified SUP-17 as a key regulator of EFF-1-mediated axonal fusion. SUP-17 is a member of the ADAM (A Disintegrin and Metalloprotease) family, and ortholog of the human metalloprotease ADAM10. *sup-17* loss-of-function leads to enhanced axonal fusion in PLM neurons. We demonstrate that SUP-17 regulates this process in a cell-autonomous fashion. We have previously shown that phosphatidylserine (PS) exposure on the damaged axon functions as a "save-me" signal to promote fusion. Our recent data show that putative PS-binding sites in SUP-17, as well as its metalloprotease activity, are essential for its function. We propose that PS exposure triggered by injury binds SUP-17 and activates its proteolytic function. Finally, biochemical analysis reveals that SUP-17 binds to both metalloprotease ADM-4 and fusogen EFF-1 to inhibit axonal fusion. Our results uncover an essential function for SUP-17 in inhibiting axonal fusion and set the foundation for designing novel therapeutics for nerve injuries.

81 GPX modulation promotes regenerative axonal fusion and functional recovery after injury through PSR-1 condensation

Su-Hyuk Ko, Zhijie Liu, Lizhen Chen University of Texas Health Science Center San Antonio

Axonal fusion represents an efficient way to recover function after nerve injury. However, how axonal fusion is induced and regulated remains largely unknown. We discover that ferroptosis signaling can promote axonal fusion and functional recovery in *C. elegans* in a dose-sensitive manner. Ferroptosis-induced lipid peroxidation enhances injury-triggered phosphatidylserine exposure (PS) to promote axonal fusion through PS receptor (PSR-1) and EFF-1 fusogen. Axon injury induces PSR-1 condensate formation and disruption of PSR-1 condensation inhibits axonal fusion. Extending these findings to mammalian nerve repair, we show that loss of Glutathione peroxidase 4 (GPX4), a crucial suppressor of ferroptosis, promotes functional recovery after sciatic nerve injury. Applying ferroptosis inducers to mouse sciatic nerves retains nerve innervation and significantly enhances functional restoration after nerve transection and resuture without affecting axon regeneration. Our study reveals an evolutionarily conserved function of lipid peroxidation in promoting axonal fusion, providing insights for developing therapeutic strategies for nerve injury.

82 TFEB/HLH-30-mediated expansion of lysosomal capacity protects *C. elegans* neurons during aging

Ruiling Zhong[1], Claire E Richardson[21]Integrative Biology, University of Wisconsin-Madison, [2]Genetics, University of Wisconsin-Madison

Lysosomes are essential for neuronal homeostasis, providing degradation and recycling functions necessary to support neurons' complex operations and long lifespans. Impaired lysosomal function is strongly implicated in the pathogenesis of neurodegenerative diseases, wherein protein aggregates – would-be cargoes of lysosomal degradation – accumulate and cause toxicity. This suggests the hypothesis that dysfunction of neuron lysosomes is a pathology of aging. Transcription factor EB (TFEB)/HLH-30 is a conserved master transcriptional activator of endo-lysosomal and autophagosomal biogenesis. TFEB/HLH-30 activity depends on its nuclear translocation, which is primarily regulated by phosphorylation status in response to starvation and stress. Whether and how TFEB/HLH-30 function feeds into the intrinsic cell biology programs within neurons to modulate homeostatic maintenance versus aging is not well understood. We find that HLH-30 is dispensable for neuronal development but acts cell-intrinsically to expand lysosomal degradative capacity during early adulthood. Loss of HLH-30 leads to lysosome dysfunction and delayed turnover of synaptic vesicle proteins from the synapse. Notably, we show that basal HLH-30 activity is sufficient to expand neuronal lysosomal capacity without nuclear enrichment, in contrast to the nuclear translocation associated with starvation- and stress-induced activation of TFEB and HLH-30. Furthermore, we show that neuronal lysosomal function declines with age in wild-type animals, and this corresponds to a decrease in basal HLH-30-mediated transcription. We demonstrate that basal HLH-30 activity is crucial for neuron maintenance: in the PVD neuron, lysosomal dysfunction due to inadequate HLH-30 activity leads to both dendrite degeneration and aberrant outgrowths, apparently through two distinct mechanisms. In summary, our study establishes a critical role for basal HLH-30/TFEB activity in promoting lysosomal capacity to preserve neuronal homeostasis and structural integrity of mature neurons *in vivo*. This work sheds light on the fundamental question of how neuron endolysosomal degradative capacity is regulated.

83 UNC-40/DCC netrin receptor stabilization leads to sex-specific, selective dopaminergic neuron degeneration

Yehuda Salzberg[1], Sapir Sela-Vasiliu[2], Rizwanul Haque[2], Hagar Setty[2], Meital Oren-Suissa[21]Brain Sciences, Weizmann Institute of Science, [2]Weizmann Institute of Science

Loss of dopaminergic (DA) neurons in adulthood is a hallmark of Parkinson's disease (PD). While disease features are manifested differently in men and women, little is known about the mechanisms that generate this dimorphism. One of the genetic loci that associate strongly with many neurological disorders, including PD, is the netrin receptor DCC, which is expressed in DA neurons of the substantia nigra in the adult mouse brain and has been suggested to be involved in PD etiology. We found that worm DA neurons are sensitive to the dose of UNC-40, and loss of UNC-40 renders them more resistant to the degeneration effects induced by the DA neurotoxin 6-OHDA. We previously generated a CRISPR strain that abrogates a conserved degron motif (UNC-40mCPD) through which the ubiquitin-proteasome system degrades UNC-40, thus over-stabilizing UNC-40. We found that hermaphrodites carrying this allele develop spontaneous degeneration of DA neurons, whereas males are resistant to the phenotype. Transgenic over-expression of UNC-40 in DA neurons is sufficient to elicit their degeneration even in males, presumably by exceeding steady-state UNC-40 levels. Thus, too much UNC-40 compromises DA neuronal health in a dose-dependent sexually-dimorphic manner. We show that the UNC-40mCPD allele likely generates stressful synaptic overload on DA neurons, eventually leading to their degeneration via the parthanatos stress-induced cell death pathway. Blocking the parthanatos effectors *wah-1* or *parp-1* or pharmacologically reducing mitochondrially-released reactive oxygen species, a known feature of parthanatos, rescues DA neuron degeneration of UNC-40mCPD animals. We further present evidence to suggest that none of this stress occurs in males due to the presence of the UNC-40 ligand UNC-6/netrin that is absent in hermaphrodites, lending support to the 'ligand-dependence receptor' hypothesis for UNC-40 function. Expectedly, dopamine-dependent behaviors are also compromised in UNC-40mCPD hermaphrodites, but not males. Transcriptome profiling of separated male and hermaphrodite populations revealed that the UNC-40mCPD allele has little effect on males, while causing a pronounced transcriptional response in hermaphrodites. Therefore, our data points to the potential involvement of DCC in PD and offers a molecular stress pathway that may underlie the recurrent association of the DCC locus with multiple Human neurological diseases.

84 Epidermal Hormone Signaling Drives Sexually Dimorphic Degeneration of Dopaminergic Neurons in Later Life

Cheng-Xi Li, Jing-Yi Peng, Jia-Hui Zhang, Xian-Ting Zeng, Xia-Jing TongShanghai Tech University

Extensive sex-specific clinical features have been widely reported for Parkinson's disease (PD), with males exhibiting a higher incidence and prevalence and showing both earlier onset and more severe motor symptoms compared to females. However, the molecular basis for these disparities remains elusive. Here, we discovered a conserved, sex-specific mechanism underlying dopaminergic (DA) neuron protection that is evident in multiple experimental models of α-synuclein-induced neurodegeneration, including *C. elegans*, *D. melanogaster*, and mice. We show that the epidermal nuclear hormone signaling is required for sex-specific neuroprotection in *C. elegans*. In hermaphrodites, epidermal nuclear hormone receptor mitigates DA neuron degeneration by promoting autophagy via the microRNA , and feminization of the male epidermis recapitulates the neuroprotective effect by elevating receptor expression. In PD model mice, sponge-RNA-based inhibition experiments demonstrate that the microRNA specifically protects DA neurons in females. Our study thus reveals a conserved, steroid hormone-driven mechanism mediating late-life neuroprotection through a microRNA, providing insights into sex differences in PD and identifying therapeutic targets for development of sex-specific interventions to treat neurodegenerative diseases.

85 Optogenetic control of TDP-43 aggregation in *C. elegans* as an ALS model

Kyung Hwan Park, Euihyeon Yu, Sooji Choi, Sangyeong Kim, Kyung Won KimLife Science, Hallym University

The pathological aggregation of TDP-43 is a hallmark of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). To investigate the mechanisms and consequences of TDP-43 aggregation, we developed an optogenetic *Caenorhabditis elegans* model using the optoDroplet system. We engineered human TDP-43 fused with Cry2olig (opto-hTDP-43) and expressed it pan-neuronally in *C. elegans*. Using this system, we achieved precise spatiotemporal control over TDP-43 aggregation, enabling the selective induction of TDP-43 phase separation and aggregation in neurons at defined timepoints using blue light illumination. Upon photostimulation, opto-hTDP-43 underwent liquid-liquid phase separation (LLPS), followed by aggregation, recapitulating key features of TDP-43 proteinopathy, including cytoplasmic mislocalization and the formation of aggregates resistant to dissolution. Despite being expressed pan-neuronally, TDP-43 aggregates induced relatively selective toxicity, with GABAergic motor neurons exhibiting heightened vulnerability, leading to severe locomotor dysfunction, characteristic of ALS. Specifically, TDP-43 aggregation resulted in impaired thrashing ability and decreased body bending frequency along with reduced exploratory behavior. Notably, while the baseline expression of TDP-43 itself exhibited some neurotoxicity, these phenotypic defects were significantly exacerbated following the induction of TDP-43 aggregation, highlighting the critical role of protein aggregation in disease progression. Additionally, TDP-43 aggregation impaired sensory processing, affecting both mechanosensation and olfaction, and shortened lifespan. This optogenetic model successfully captures key aspects of TDP-43 pathology in a neuronal system. It provides a powerful platform to investigate disease mechanisms and screen to potential therapeutic interventions targeting TDP-43 aggregation in neurodegenerative diseases.

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86 Regulation of local translation of Flippases by OMA-1/ZFP36L is required for axon degeneration

Zhongwei Qu MGM, Duke University Medical Center

Axon degeneration is a hallmark of neurodegenerative diseases and aging, with NMNAT and Sarm1 identified as key regulators of axon degeneration. However, the exact underlying mechanisms remain incompletely understood. *C. elegans* has been widely used as a model organism in neurobiology. However, the prevailing belief that *C. elegans* lacked Wallerian degeneration or employed different mechanisms to regulate axon degeneration compared to *Drosophila* and mice. Here, we challenge this notion by demonstrating that the neurons of adult *C. elegans* do undergo Wallerian degeneration, but with a slower speed. In this study, we show that severed axons exhibit progressive degeneration over days, mirroring morphological features observed in other species. Furthermore, we provide genetic evidence that *C. elegans* employs the conserved NMNAT-Sarm1 pathway to drive axon degeneration, establishing it as a suitable model for studying this process. Using *C. elegans as the model organism*, we identify *C. elegans* OMA-1 and its mammalian homologs, ZFP36L1 and ZFP36L2, as novel mediators of axon degeneration acting downstream of Sarm1. OMA-1/ZFP36L regulates the mRNA decay of the conserved flippase TAT-2/ATP8B2, suppressing its local translation. The absence of TAT-2 in injured axons promotes degeneration, highlighting the critical role of local mRNA regulation in axon integrity. Our findings not only indicate *C. elegans* as a powerful model organism for studying axon degeneration but also uncover a conserved new mechanism governing axon degeneration.

87 Investigating lysosomal-autophagy pathway dysfunction in a *C. elegans* model of tau and TDP-43 synergy

Heino Hulsey-Vincent[1], Vaishnavi Jadhav[2,3], Paul Valdmains[2], Brian Kraemer[1,2,3], Nicole Liachko[2,3], Caitlin S Latimer[11]Laboratory Medicine and Pathology, University of Washington, [2]Medicine, University of Washington, [3]Geriatric Research Education and Clinical Center, VA Puget Sound Health Care System

Although Alzheimer's disease (AD) is pathologically defined by the presence of both amyloid plaques and tau neurofibrillary tangles, TDP-43 pathology often occurs comorbidly with AD and correlates with more rapid cognitive decline and faster rates of hippocampal atrophy. We have previously shown that there is a synergistic relationship between tau and TDP-43 in a novel *C. elegans* model of combined tau and TDP-43 pathology and that the addition of TDP-43 may enhance tau proteotoxicity. However, the mechanisms that drive this interaction are unknown. To further explore this relationship, we evaluated transcriptomic changes at time-points preceding frank neuronal loss in our *C. elegans* model of tau and TDP-43 co-expression (tau+TDP-43 Tg). We then followed up our findings with functional assessments in *C. elegans* to test the role of specific genes in promoting or protecting against tau+TPD-43 synergistic proteotoxicity.

In the *C. elegans* transcriptomic analyses we found significant differential expression of genes enriched in the lysosomal-autophagy pathway Genes in this pathway had increased expression in worms expressing human tau compared to wildtype. This is consistent with some human studies, which found a compensatory upregulation of the lysosomal-autophagy pathway in early AD. However, in worms expressing tau and TDP-43 this compensatory upregulation was impaired. One of the most significantly differentially expressed genes with a human homologue was *cpr-8 (cathepsin-B),* a lysosomal enzyme involved in protein degradation. Subsequent function studies revealed that loss of cpr-8 function phenocopied the tau+TDP-43 phenotype in the tau strain. Overall, these results highlight the relevance of lysosomal-autophagic dysfunction in the synergistic proteotoxicity of tau and TDP-43, suggesting this combination of pathologic proteins may undermine cellular resilience to tau pathology in neurons.

88 Potassium Homeostasis Regulates Presynaptic Organization Independent of Membrane Potential

Jun Meng1,2, Neeraja Ramakrishnan3, Yi Li4, Ben Mulcahy5, Yan Li5, Thamos Boulin6, Mei Zhen5, Isabel Beets1, William Schafer1,21KU Leuven, 2MRC Laboratory of Molecular Biology, 3University of Toronto, 4Shenzhen Bay Laboratory, 5Lunenfeld-Tanenbaum Research Institute, 6Université Claude Bernard Lyon 1 Synaptic organization is fundamental to neural circuit function, yet the underlying regulatory mechanisms remain incompletely understood. While neuronal activity and membrane potential are well-established regulators of synaptic assembly, alternative mechanisms governing activity-independent pathways remain unclear.

Here, we identify a membrane potential-independent mechanism by which intracellular potassium homeostasis, regulated by a two-pore domain potassium (K2P) channel, TWK-40, controls presynaptic organization in *C. elegans* cholinergic premotor interneuron AVA. Specifically, we find that loss of TWK-40 function results in excessive accumulation of presynaptic proteins, whereas gain-of-function mutations deplete presynaptic components, leading to synaptic transmission deficits. The channel itself is presynaptically localized and modulates synaptic vesicle organization and neurotransmitter release. Surprisingly, using live imaging, genetic perturbations, and functional assays, we observed that these effects are independent of the channel›s effects on excitability or pH, but correlate with effects on intracellular K+. Mechanistically, we find that the K2P channels maintain intracellular potassium homeostasis, which in turn regulates a transcriptional network involving terminal selectors that control actin-associated proteins essential for synapse dynamics. Our findings reveal a noncanonical, genetically encoded, activity-independent transcriptional program that cholinergic premotor interneurons utilize to maintain presynaptic organization.

89 Building the Cell Observatory: Project Worm

David Q Matus, Gokul Upadhyayula, Eric Betzig Molecular and Cellular Biology, University of California Berkeley

Development is an emergent process that requires complex orchestration across scales, from the genomic, epigenetic, molecular, and cellular to tissue level dynamics. While reductionist approaches have been very successful at teasing out mechanistic answers to specific cell and developmental processes, to truly understand development *in toto* we need to build tools to capture and analyze developmental biology at a sub-cellular level, in living organisms, as it happens, in real time. To accomplish this task, we are combining expertise in transgenic line generation and endogenous tagging at native loci, state-of-the-art light sheet microscopy and innovations in artificial intelligence with the goal of building a foundational model for interrogating developmental dynamics in vivo. While the creation of this Cell Observatory is a multi-year effort spanning several model organisms, we are currently generating the first adaptive optics lattice light sheet microscopy (AOLLM) datasets for the project. We are leveraging the many strengths of *C. elegans* as a model system to quickly iterate on transgenic line generation to achieve our first goal of accurate cell segmentation in four dimensions. We will discuss our progress on adapting and benchmarking new photostable fluorescent proteins to robustly label cell membranes and nuclei and recombination strategies to generate multicolored labeling to assist in cell segmentation, tools instrumental to the project as well as useful to the *C. elegans* community at large.

90 Deep-learning based automated reconstruction of anatomical axis coordinates in developing *C. elegans* larvae

Gouri Patil, Jeroen van Zon AMOLF

Animal bodies are organised along anatomical axes, both body-wide, such as anteroposterior (AP) and dorsoventral (DV) axes, and within organs, such as proximal-distal axes. These axes are of utmost biological importance, as they align with underlying molecular gradients, for instance of WNT or netrin proteins. In this way, these axes act as coordinate systems that instruct cells on their position within the body, to control cell division or differentiation programs, or their direction of movement, in case of migrating cells. Elucidating developmental processes along body axes is notoriously hard, even in simple animals like *C. elegans*, as it requires manual annotation of axes and cell position. While deep-learning based neural networks are highly successful in automated recognition of body posture in complex animals, they function by recognizing discrete points of interest, such as snout tips and limb joints, and cannot be extended to anatomical axes, which instead vary continuously throughout the body. Here, we use deep-learning neural networks to predict 'anatomical fields', i.e. continuous maps of AP and DV position, based on complex transmitted light microscopy images. Using time-lapse microscopy data of developing *C. elegans* larvae, we show that our approach readily segments the animal's body and generates highly reliable AP and DV maps, even as the animal's anatomy changes strongly during larval development. AP and DV maps were generated not only with the correct orientation, but were sufficiently uniform to function as accurate AP and DV coordinate systems. We show two applications that demonstrate the power of our approach. First, we use automated body segmentation and detection of AP-DV orientation to quantify the timing of body growth and lethargus-specific 'flipping' of larvae along their AP-axis. Second, we use AP and DV maps to provide fully automated quantification of cell migration. We specifically focus on migration of distal tip cells (DTCs), which specify gonad shape by a complex and precisely-timed sequence of migrations along the AP and DV axis. Here our analysis enables automated quantification - across dozens of animals - of migration errors, changes in time and migration speed, including in mutants of netrin genes such *unc-5*, *unc-6* and *unc-40* that perturb DTC migration. Given its generality, our approach will enable anatomical axes reconstruction also within organs and in other animals.

91 Single-cell-RNA-sequencing reveals novel cell- and non-cell-autonomous roles for a conserved terminal selector gene

Jayson J Smith[1], Seth R Taylor[2], Grace S Kim[3], John G White[4], David H Hall[3], David M Miller[5], Paschalis Kratsios[11]Department of Neurobiology, University of Chicago, [2]Brigham Young University, [3]Albert Einstein College of Medicine, [4]MRC Laboratory of Molecular Biology, [5]Vanderbilt University

Neuron type identities are established during development and maintained throughout life by a class of transcription factors (TFs) known as terminal selectors. Originally identified in the nervous system of *C. elegans*, terminal selectors have since been identified throughout the model organisms. Despite their critical role in nervous system development and their direct links to various human neurodevelopmental conditions, our understanding of the molecular mechanisms underlying terminal selector functions has largely been confined to insights from biased, candidate-gene studies focused on cell-autonomous effects.

To gain novel molecular insights, we employed an unbiased approach using single-cell RNA sequencing (scRNA-seq) to study the terminal selector UNC-3 (Collier/Olf/Ebf) in motor neurons (MNs) of the *C. elegans* ventral nerve cord, examining both cholinergic (UNC-3[+]) and GABAergic (UNC-3[-]) populations. In cholinergic MNs, loss of UNC-3 disrupts the transcriptional identities of eleven distinct subclasses, reducing them to seven mutant MN groups. These groups acquire new molecular characteristics, including alternative neurotransmitter identities. The single-cell resolution of RNA-Seq enabled us to make a striking observation: loss of the same TF (UNC-3) has different effects in different MN types. That is, in the *unc-3(-)* mutant, the identities of four MN classes (DA, DB, VA, VB) collapse into three distinct mutant populations, whereas both class- and subclass-specific identity features are partially retained in another MN class (AS).

By integrating our scRNA-seq dataset with UNC-3 ChIP-seq analysis, we identified hundreds of novel UNC-3-activated targets and uncovered a previously unrecognized role for the TF: it actively represses—likely directly—genes associated with inappropriate neuron type-specific identities in cholinergic MNs. Using the Auxin-Inducible Degron (AID) system to deplete UNC-3 late in development, we further demonstrated that this repressive function persists throughout life. Altogether, our data suggests that UNC-3 performs a dual transcriptional activator-repressor role to establish and maintain cholinergic MN terminal identities in a type-specific manner.

Unexpectedly, we found that loss of UNC-3 in cholinergic MNs also triggers non-cell-autonomous effects, altering the transcriptomes, morphology, and connectivity of postsynaptic GABAergic MNs. Overall, the resolution of scRNA-seq enabled us to uncover new cell-autonomous and non-cell-autonomous roles for UNC-3, suggesting such regulatory mechanisms might underpin the functions of TFs in other neuron types and/or developmental contexts.

92 A genetic approach to identifying egg specific fertilization genes and the role of glycans in this process

Katherine A. Maniates, Kendall Ann Flanagan, Shania Halim, Zahra Mohammad, Andrew Singson Waksman Institute, Rutgers University

Fertilization and the activation of gametes are precise events that require cell-cell adhesion, binding, fusion, signaling, and recognition. Despite the importance of fertilization for all sexually reproducing organisms, many of the genes and molecules involved in generating the fertilization synapse are still unknown. In particular, many fewer genes and molecules on the egg have been identified as being involved in fertilization. The asymmetry between known sperm and egg components required for fertilization is seen across many organisms including mice, flies, worms, and fish. Identification of these genes required in the egg for fertilization has been challenging due to both biological and technical reasons, among these reasons are redundancy, pleiotropy, and the complexity of these processes. Many of these challenges are not unique to identifying genes involved in fertilization, but also in any field that encounters sterile or lethal mutant phenotypes.

We have developed a forward genetic screening approach using the Mitani lab's recently developed CRISPR/Cas9 engineered balancer chromosomes to identify new genes required for fertilization. This approach is valuable for researchers across fields that are searching for sterile or lethal mutants. One significant gene that we identified is *egg-7,* an ortholog of mammalian phosphoglucomutase 3 (PGM3). PGM3 is an enzyme involved in glycosylation and synthesis of GPI anchors. Mutants in this gene are egg-specific sterile, hermaphrodite animals have normal morphology and germline development. However, these mutants are sterile, and fertility is unable to be recovered after mating with males. *egg-7* mutants also have decreased levels of lectins on the egg surface consistent with its role in glycosylation. EGG-7 is localized in the oogenic germline and early embryos. We hypothesize that *egg-7* is required for glycosylation and synthesis of GPI anchor proteins on the oocyte's surface for fertilization. As homologues of EGG-7 in vertebrates have broad expression in somatic tissues. We have now established a germline specific model to understand what happens in the gametes with the loss of PGM-3/EGG-7 without the complication of somatic phenotypes.

93 Evolutionary diversification of actomyosin-driven early embryo morphogenesis

Jacobus van Grootel[1], Mario AM Monleon[1], Teije C. Middelkoop[21]Institute of Molecular Genetics of the Czech Academy of Sciences, [2]Institute of Molecular Genetics

The shape of an animal arises gradually during embryonic development in a process referred to as 'morphogenesis'. In nematodes, morphogenesis starts immediately after fertilization and requires a tight coordination of cell polarization, cell division and cellular-scale rotations. Biophysical studies have shown that all these processes depend on active forces and torques generated in the actomyosin cytoskeleton of embryonic cells. In recent years, much has been learned about the force-generating mechanisms that drive early embryo morphogenesis in C. elegans. However, very little is known about whether and how these physical mechanisms have diversified during nematode evolution. To study this, we analyzed actomyosin-driven morphogenesis in a panel of related nematode species. We performed time lapse imaging of polarization and cell divisions up until the 4-cell stage. Subsequently, we quantitatively characterized intracellular actomyosin dynamics as well as cellular-scale morphogenetic rotations. We find striking differences in the dynamic behavior of the actomyosin cytoskeleton. For example, in C. elegans the actomyosin cytoskeleton undergoes intracellular chiral rotations that, in turn, drive cellular rotations. This rotatory actomyosin behavior was variable among species, with some displaying no rotatory behavior at all. Nonetheless, in agreement with earlier studies, we find that the overall pattern of polarization and cell orientation displays little variability among the nematodes analyzed. Altogether, these results are indicative of developmental systems drift, where different physical mechanisms give rise to a similar developmental outcome.

94 Regulation of cell polarity orientation by expression gradients of Wnt receptors

Misato Matsuo, Masayo Asakawa, Hitoshi Sawa National Institute of Genetics

Proper regulation of cell polarity orientation plays essential roles in animal development. Although Wnt signaling is pivotal in polarity regulation during development of animals, roles of Wnts are not clear yet. Although Wnt gradients have been suggested to instructively control polarity orientation mostly by ubiquitous Wnt expression experiments, the effects may be due to overexpression rather than uniform Wnt expression. During development of *C. elegans,* most cells are polarized along the anterior-posterior axis and divide asymmetrically. We have shown that multiple Wnt proteins redundantly controls polarity orientation of seam cells, since triple Wnt mutants showed random polarity orientation. Since such phenotype can by rescued by Wnt ectopic expression that reverses the Wnt gradients, Wnt functions appear to be permissive. We have further found that, in the background of *lin-17*/Frizzled mutants, similar ectopic Wnt expression enhanced polarity reversal in triple Wnt mutants. These results indicate that Wnts have both instructive and permissive functions controlling polarity orientation.

To understand how Wnt permissively control polarity, we focused on expressions of Wnt receptors as possible cues. Polarity of seam cells are redundantly controlled by three receptors, MOM-5/Fzd, LIN-17/Fzd and CAM-1/Ror that show graded expression along the body axis. MOM-5 and CAM-1 are higher in the anterior side, while LIN-17 is higher in the posterior side. We found that the expression of CAM-1 using the *lin-17* promoter (*lin-17p*::CAM-1) reversing its expression gradient, caused seam cell polarity defects. The defects were enhanced by mutating *cam-1*, suggesting that endogenous *cam-1* and *lin-17p*::CAM-1 have antagonistic functions. Furthermore, seam cell polarity was strongly reversed by *lin-17p*::CAM-1 in *mig-14*/Wntless mutants decreasing Wnt concentration and possibly inhibiting instructive function of Wnts. In addition, reversing MOM-5 expression gradients using *lin-17p*::MOM-5 in *cam-1* mutants caused strong polarity reversal. The results indicate that expression gradients of Wnt receptors function as cues of polarity orientation of asymmetrically dividing cells.

95 The Raf/LIN-45 C-terminal distal tail segment negatively regulates signaling in *Caenorhabditis elegans*

Robert A Townley[1], Kennedy S Stacy[1], Fatemeh Cheraghi[1], Claire de la Cova[21]Biological Sciences, University of Wisconsin-Milwaukee, [2]University of Wisconsin-Milwaukee

Raf protein kinases act as Ras-GTP sensing components of the ERK signal transduction pathway in animal cells, influencing cell proliferation, differentiation, and survival. In humans, somatic and germline mutations in the genes *BRAF* and *RAF1* are associated with malignancies and developmental disorders. Recent studies shed light on the structure of activated Raf, a heterotetramer consisting of Raf and 14-3-3 dimers, and raised the possibility that a Raf C-terminal distal tail segment (DTS) regulates activation. We investigated the role of the DTS using the *Caenorhabditis elegans,* which has a single Raf ortholog termed *lin-45*. We discovered that truncations removing the DTS strongly enhanced *lin-45(S312A)*, a weak gain-of-function allele equivalent to *RAF1* mutations found in patients with Noonan Syndrome. We generated mutations to test three elements of the LIN-45 DTS, which we termed the active site binding sequence (ASBS), the KTP motif, and the aromatic cluster. In the context of *lin-45(S312A),* mutation of either the ASBS, KTP motif, or aromatic cluster enhanced activity. We used AlphaFold to predict DTS protein interactions for LIN-45, fly Raf, and human BRAF, within the activated heterotetramer complex. We propose distinct functions for the LIN-45 DTS elements: i) the ASBS binds the kinase active site as an inhibitor, ii) phosphorylation of the KTP motif modulates DTS-kinase domain interaction, and iii) the aromatic cluster anchors the DTS in an inhibitory conformation. This work was recently published [1]; our findings establish that the Raf DTS negatively regulates Raf signaling in *C. elegans* and provide a model for Raf function in humans.

1. Townley RA, Stacy KS, Cheraghi F, de la Cova CC. The Raf/LIN-45 C-terminal distal tail segment negatively regulates signaling in *Caenorhabditis elegans*. Genetics. 2024 Nov 6;228(3):iyae152. doi: 10.1093/genetics/iyae152.

96 PP1cb functions with myosin phosphatase-targeting subunits to promote anterior enclosure during embryogenesis

Neha Varshney[1], Rebecca Green[1], Sindy Chavez[1], Arshad Desai[1,2], Karen Oegema[1,21]Department of Cell & Developmental Biology, University of California San Diego, [2]Department of Cellular and Molecular Medicine, University of California San Diego

Protein phosphatase 1 (PP1c) regulates multiple cellular processes by targeting a diverse set of proteins. The mechanisms that determine PP1c specificity—ensuring it acts on the right targets at the right time and place—are an active area of research. In this study, we investigate the role of PP1c during embryonic development in *C. elegans*, where two catalytic isoforms, PP1cbGSP-1 and PP1caGSP-2, are active. These isoforms share ~90% sequence identity, are expressed at similar levels, and function largely redundantly during the early embryonic cell divisions. However, PP1cbGSP-1 depletion—but not PP1caGSP-2—causes embryonic lethality, suggesting a non-redundant, essential function for PP1cbGSP-1 during the later stages of embryogenesis. Live imaging revealed that PP1cbGSP-1 is dispensable for germ layer specification but is required for anterior enclosure, the process in which the epidermis encloses the forming head. Imaging of GFP::PHA-4 revealed defects in pharynx structure and anterior attachment, supporting a role for PP1cbGSP-1 in coordinating anterior morphogenesis. Using 2-hybrid analysis, we found that the MYPT family proteins—MEL-11 (MYPT1) and GFI-2 (MYPT3)— act as PP1cbGSP-1-specific targeting subunits. Depleting MEL-11 resulted in an anterior enclosure defect that phenocopied PP1cbGSP-1 depletion, while GFI-2 depletion resulted in a similar but less penetrant phenotype. Using auxin-inducible degradation, we found that elimination of maternally-provided PP1cbGSP-1 did not result in a head rupture phenotype but revealed strong zygotic expression of PP1cbGSP-1 in the skin, pharynx, intestine and neurons. Tissue-specific degradation of PP1cbGSP-1 in pharyngeal cells partially recapitulated the anterior enclosure defect, while degradation in neuronal precursor cells fully recapitulated it, suggesting that PP1cbGSP-1 functions in both neuronal precursor and pharyngeal cells. Together, these results highlight a critical role for PP1cbGSP-1 in anterior morphogenesis, where it partners with MYPT1 and MYPT3 to provide spatially-patterned myosin phosphatase activity. These findings have the potential to provide insight into Noonan-like syndrome, a rare human congenital disease linked to mutations in PP1cβ.

97 Insulin signaling acts in the somatic gonad to regulate dauer entry in *C. elegans*

Julia Sarah Wittes[1], Grace Przybyl[2], Iva Sarah Greenwald[21]Biological Sciences, Columbia University, [2]Department of Biological Sciences, Columbia University

In response to adverse environmental conditions, *C. elegans* larvae can pause reproductive development and enter dauer diapause. The decision to enter dauer or develop continuously involves insulin/insulin like signaling (IIS), TGF-β, and nuclear hormone receptor signaling, but the tissues where each pathway functions and how pathway activities are integrated to influence dauer entry are incompletely understood.

We developed new Flexon-based reagents to disrupt gene activity in the somatic gonad using the auxin-inducible degron (AID) system or tissue-specific RNAi (Shaffer et al., 2022; Wittes and Greenwald, 2024). Previous genetic studies suggested that IIS might have a diffuse tissue focus for regulating dauer entry; for instance, tissue-specific AID of the insulin receptor DAF-2 in a variety of individual tissues did not result in dauer entry in standard laboratory conditions (Roy et al., 2022; Zhang et al., 2022). Using our new reagents, we discovered that IIS activity in the somatic gonad regulates the dauer entry decision. Dauer entry can be produced at high penetrance by somatic gonad-specific AID of the insulin receptor DAF-2 or somatic gonad-specific RNAi targeting AKT in a DAF-2(+) background, which acts at an intermediate step in IIS. By varying the timing of auxin addition or removal in experiments conducted with a DAF-2::AID allele, we confirmed that DAF-2 acts in the L1 stage to mediate the L2/L2d decision and during L2d to mediate the L3/dauer decision. Furthermore, constitutive dauer entry caused by low IIS in the somatic gonad requires *daf-16/* FOXO activity within the somatic gonad. Preliminary results from laser ablation experiments suggest that the germline mediates communication between the somatic gonad and the non-gonadal soma.

The surprising finding that IIS activity in the somatic gonad plays a critical role in the dauer entry decision demonstrates that the gonad is a key endocrine organ and regulates development at the whole-organism level. We will discuss potential models for the interorgan signaling that regulates dauer entry in light of these new findings.

98 Formylglycine and an alternative maturation system regulate nematode sulfatases

Catia Igreja[1], Radhika Sharma[1], Tobias Loschko[1], Hanh Witte[1], Johannes Maldung[2], Mirita Franz-Wachtel[2], Boris Macek[2], Ralf Sommer[11]Department for Integrative Evolutionary Biology, Max Planck Institute for Biology, [2]Interfaculty Institute of Cell Biology, University of Tübingen

Sulfatases hydrolyze sulfate esters during hormone synthesis or the catabolism of sulfated sugars and lipids. Sulfatase activity resides in a conserved formylglycine (FGly) residue, co-translationally generated in the endoplasmic reticulum (ER) by the modification of a cysteine (Cys). In all domains of life, FGly modifications are essential for sulfatase activity. Mutations impairing the function of the human FGly-generating enzyme (FGE), lead to Multiple sulfatase deficiency (MSD), a rare and fatal disease. Efforts to understand desulfation in worms are restricted to the neuronal sulfatases *C.elegans* SUL-2 and its paralog EUD-1 in *Pristionchus pacificus*. *eud-1* is the switch gene guiding the developmental decision underlying the mouth-form and feeding polyphenism in *P. pacificus*. Strikingly, the catalytic Cys is conserved in nematode sulfatases; yet, homology searches indicate that FGE homologs are not present in nematodes, thus, suggesting an alternative FGly-generating system with an elusive FGE. Here, we present our quest for the missing nematode FGE. We generated CRISPR-edited worms encoding *eud-1* versions lacking the signal peptide or with a Cys to alanine (Ala) substitution on the active site. We observed that EUD-1 ER import and the catalytic Cys are crucial for the role of the sulfatase in mouth dimorphism. To elucidate whether nematode sulfatases are modified to FGly, we expressed and purified EUD-1 in *E. coli* in the presence of a heterologous FGE. Detection of the FGly residue by LC-MS/MS and chemical conjugation indicated that the catalytic Cys was efficiently modified to FGly. We also investigated the presence of FGly in endogenously tagged sulfatases. Immunoprecipitation of ALFA-tagged SUL-2 from extracts of wild-type or Cys to Ala-edited worms, coupled with FGly detection, provided the first evidences that the active site Cys is also modified to FGly in worms. Thus, nematodes express an unknown enzyme that in the ER modifies to FGly the sulfatases active site. We will present our efforts to identify this enzyme using proximity biotinylation and immunoprecipitation assays paired with proteomics.

99 Ras switching from Raf to RalGEF during VPC induction: basal vs. apical recruitment

Rudra Prasana Banerjee[1], Neal Rasmussen[1], Hanna Shin[2,3], Asenath Genawnaw[4], Guadalupe Calderon[3], David Reiner[1,41]Institute of Biosciences and Technology, Texas A&M Health Science Center, [2]Texas A&M Health Science Center, [3]College of Medicine, Texas A&M University, [4]Texas A&M University

The Ras small GTPase is the most frequently mutated oncoprotein, activating three main effector pathways: Raf>MEK>ERK, PI3K>PDK>Akt and RalGEF>Ral. Raf and PI3K are well-studied and pharmacologically targeted, but RalGEF>Ral is less understood. Ras-positive tumors can switch effectors but the mechanism is unknown.

The induction and patterning of vulval precursor cell (VPC) fates in *C. elegans* provides a model for investigating Ras signaling. EGF induces six VPCs to form the 3˚-3˚-2˚-1˚-2˚-3˚ pattern of cell fates with 99.8% accuracy. The EGF Receptor in the basal VPC compartment is necessary for inducing the 1˚ cell fate; without it, animals are Vulvaless.

We previously showed that Ras switches effectors during VPC fate patterning, from Raf (1˚) to RalGEF>Ral (2˚). Activated Ral then signals through the EXOC-8/Exo84>GCK-2/CNH-MAP4K>MLK-1/MAP3K>PMK-1/p38 MAP Kinase pathway to promote the 2˚ fate.

Using CRISPR, we tagged endogenous LIN-45/Raf and RGL-1/RalGEF with a yellow-green fluorescent protein (LIN-45::mNG and mNG::RGL-1) and LET-60/Ras with a red fluorescent protein (mS::LET-60). As expected, mS::LET-60 localized to all plasma membranes due to its C-terminal lipid modification. LIN-45::mNG and mNG::RGL-1 exhibited striking subcellular localization in VPCs. Upon induction, LIN-45::mNG was recruited to the basal plasma membrane in presumptive 1˚ cells, while mNG::RGL-1 was recruited to the apical compartment in two phases. Initially, mNG::RGL-1 was recruited apically in all VPCs, but its recruitment decreased in presumptive 1˚ cells and persisted in 2˚ cells.

We introduced a human RA-defective mutation into mNG::RGL-1, abolishing its apical recruitment. We also edited the T35S mutation, which supports Ras>Raf but abolishes Ras>RalGEF signaling, into LET-60/Ras. T35S animals failed to recruit mNG::RGL-1 apically. Recruitment of tagged 2˚-promoting Ral effector, Exo84/EXOC-8::mNG mirrored that of mNG::RGL-1 but was more consistent and brighter.

These findings suggest that Ras switches effectors by compartmentalization along two spatial axes: apical-basal and anterior-posterior (*i.e.* 1˚ vs. 2˚). The basal compartmentalization of LIN-45::mNG may relate to the requirement for basal EGFR in vulva induction.The mechanism behind apical compartmentalization of mNG::RGL-1 or segregation of 1˚ and 2˚ signals remains unclear. This study highlights a key Ras signaling phenomenon that may inform therapeutic strategies for Ras-positive tumors.

100 Neighboring tissues align apical surfaces via cell-cell contact and HMR-1-mediated signaling to enable continuous digestive tract formation

Lauren Cote[1], Maria Sallee[2], Jessica Feldman[1]1Biology, Stanford University, [2]Buck Institute

Epithelial cells form a continuous barrier with specialized apical surfaces facing a lumen or the external environment, yet how neighboring tissues join and align apical surfaces remains understudied. An epithelial toroid consisting of the digestive tract and the skin form a protective epithelial barrier that encases all internal tissues, however these tissues arise and undergo apical polarization at different times and locations during embryogenesis. The *C. elegans* digestive tract, with the intestine forming and polarizing near the dorsal surface of the embryo and the rectum forming ventrally, is an in vivo model for understanding how neighboring epithelial tissues connect. Our work has established that the two rectal valve cells play a critical role in choreographing the rectal-intestinal connection. The valve cells initially migrate from the ventral side of the embryo to the posterior end of the intestine where they induce the intestine to form an open tube and not a closed-off cyst. Laser ablations of the rectal valve cell progenitor resulted in a cystic intestine that was not connected to the rectum due to a failure of the most posterior intestinal cell to complete apical polarization. Conversely, the intestine is required to properly polarize the valve cells, which in turn allows the valve to align and connect the apical surface of the intestine with that of the rectum. We found that the valve cells initially polarize by each forming bipolar puncta of apical and junctional proteins with one anterior punctum contacting the apical surface of the intestine and one posterior punctum contacting the apical surface of the rectum. The anterior puncta require contact with the intestine as this structure does not form following the ablation of the posterior intestine. Contact alone, however, does not appear to be sufficient for valve polarity as intestine-specific depletion of HMR-1/E-cadherin resulted in a loss of the anterior valve punctum and a redistribution of apical and junctional proteins to the posterior valve punctum. This redistribution of apical components within the valve cells does not occur in embryos with intestine-specific depletion of PAR complex components yet can occur when intestinal cells are forced to remain in mitosis. We have therefore shown that cell-cell contact is necessary but not sufficient to correctly polarize this neighboring tissue and that proper polarization across tissue boundaries requires HMR-1-mediated signaling to create a continuous lumen. Understanding how one tissue can instruct the apical polarization of the neighboring tissue is of crucial importance to multiple contexts, including development of major organ systems, progression of diseases like polycystic kidney disease, and colonization of new tissues by metastatic cells of epithelial origin, which all require careful interplay of adhesion at the interface of two tissues.

101 *Steinernema hermaphroditum* is a genetically tractable nematode with a highly plastic body size and cell number

Chieh-Hsiang Tan[1], Nathan Y Rodak[1,2], Paul W Sternberg[1]1Division of Biology and Biological Engineering, California Institute of Technology, [2]Current Address: Case Western Reserve University

Animals with similar anatomy can have very different body sizes. Nematodes are a prime example of this: while most nematodes measure about 1 mm or smaller in length, many animal-parasitic nematodes are much larger, with some parasites of humans reaching over a meter in length. Size differences can also be observed within the same animal species, especially when they develop under different environmental conditions. The underlying mechanisms that regulate the size of an individual or that coordinate growth among body parts are fundamental questions in developmental biology with extensive implications for human health.

We identified the clade IV entomopathogenic nematode *Steinernema hermaphroditum* as a promising animal model to address these questions. Like many other parasitic nematodes, *S. hermaphroditum* adults are much larger (can reach over 5 mm in length) than that of *Caenorhabditis elegans* but can be cultured using similar conditions and offer many of the same advantages for molecular genetics research. We found *S. hermaphroditum* to be incredibly size flexible: adult animals raised under different culture conditions can differ up to nearly 100 times in volume. Unlike in *C. elegans*, the body size differences are not solely due to differences in cell size; we observed that larger animals have more somatic cells.

To understand how body size is genetically controlled, we developed an efficient co-CRISPR strategy to knock out genes of interest. Loss-of-function mutations in the ortholog of *C. elegans dbl-1* (Dpp/BMP-like), a key component of the TGFβ pathway, had very little effect on the size of *S. hermaphroditum*, in sharp contrast to the effects of *dbl-1* loss-of-function mutations in *C. elegans*. Knocking out a Hippo gene *Sthmcst-1,* the ortholog of *C. elegans cst-1/2*. altered the size of the worm significantly, an effect not reported in *C. elegans cst-1/2* mutants. *Sthm-cst-1* mutants were smaller than the wild type, in contrast to the pro-growth phenotypes described in *hippo* mutants of *D. melanogaster*. Our results from generating mutants of the *S. hermaphroditum* orthologs of *dbl-1*/TGFβ and *cst-1*/Hippo demonstrate that the genetics of size scaling differs greatly between *S. hermaphroditum* and *C. elegans*.

To study how body size is regulated by the environment, we developed methods to culture *S. hermaphroditum* consistently in agar media and liquid culture. To systematically characterize how the availability of resources or population density affects the worm's development, growth, and body size, we developed a simple but highly effective axenic liquid culture protocol that allows us to precisely manipulate nutrient levels. We have found that adult body size and somatic cell numbers are correlated with nutrient levels and that *S. hermaphroditum* appears to lack the starvation-induced early larval arrest found in *C. elegans*.

102 The retention landscape of interspecies hybridization is driven by selfish elements in minor genome

Yanwen Shao, Yiwen Zhang, Runsheng Li City University of Hong Kong

Hybrid incompatibility (HI) between closely related species poses significant barriers to gene flow and complicates our understanding of genomic interactions. *Caenorhabditis nigoni* and *Caenorhabditis briggsae* are two sister species that serve as valuable models for studying HI, as they produce viable F1 hybrids. Hybrid males are either lethal or sterile, which is one of the main reasons for blocking the gene flow and limiting the genomic comparison of two species within one organism. In this study, we successfully generated the first intermediate species, LRS129, through backcrossing transgenic *C. briggsae* individuals to *C. nigoni*. Possessing *C. nigoni* background with a *C. briggsae* X Chromosome fragment, LRS129 populations maintained a stable homozygous genotype. LRS129 females were capable of crossing with *C. briggsae* males, resulting in fertile F1 hybrids of both sexes. Utilizing the F1 hybrids, we conducted long-term evolution experiments, which revealed that *C. nigoni* X chromosome and autosomes rapidly dominated the hybrid genomes. *C. briggsae* haplotype was largely purged, with fragments of autosomes retained. The persistence of these *C. briggsae* autosome fragments was attributed to the selfish genetic elements. This is the first laboratory study to obtain an animal intermediate species and examine the equitable genomic competition between two species. We are also the first study to initiate the long-term interspecies gene flow in diploid organisms. Our study provides a significant advancement in the field of interspecies reproduction.

103 Non-uniform inheritance of mtDNA among wild isolates of *C. elegans* reveals natural variation in mitochondrial purifying selection

Samantha Fiallo, Kristin Bartlett, Ethan Berks, Cricket Wood, Pradeep Joshi, Joel Rothman UC Santa Barbara

Eukaryotic cells typically contain many copies of mitochondrial (mtDNA), meaning that different mtDNA sequences can exist within a given cell, a condition called heteroplasmy. Mitochondrial DNA (mtDNA) is susceptible to damage from reactive oxygen species produced by oxidative phosphorylation, which, if uncorrected, can result in debilitation or lethality. Cells have therefore evolved the process of mitochondrial purifying selection (MPS), in which mitochondria with defective genomes are selectively removed. Deleted mtDNA genomes can outcompete intact mtDNA owing to their replicative advantage, as is seen for the stably heteroplasmic 3.1 kb mtDNA deletion, *uaDf5*, the dominant species (~72% of total mtDNA) in animals carrying it. We previously reported that *uaDf5* progressively accumulates with age in *C. elegans* N2 hermaphrodites and that germline programmed cell death regulators are necessary for MPS of this deleted mtDNA (Flowers *et al*, *eLife*, 2023). To test the hypothesis that lab domestication might have led to relaxed MPS in the N2 strain, we tested whether natural wild isolates vary in their ability to stably maintain *uaDf5*. Using the GPR-1 overexpression system, we created cybrid lines containing fully paternal nuclear DNA (nDNA) derived from wild isolates covering the full divergent set, and fully maternal N2 mtDNA bearing the defective *uaDf5*. We found that while most wild isolates maintain *uaDf5* at levels similar to those seen in N2, two wild isolates, CX11314 and EG4725, give rise to heterogeneous populations, showing either stable, N2-like heteroplasmy, or levels that vary widely between siblings. Some of these cybrid lines completely eliminated *uaDf5*, suggesting that they may possess a more robust MPS system. We found that this non-uniform inheritance of mtDNA is not attributable to selection pressures induced by nutrient deprivation. We observed that mitochondrial activity detected with TMRE staining is reduced in early N2 embryos bearing *uaDf5* compared to those with only intact mtDNA. In contrast, embryos derived from CX11314; *uaDf5* cybrid lines show sustained high mitochondrial activity, irrespective of deficient mtDNA burden, suggesting that this strain compensates more effectively for defective mtDNA. Further, our preliminary analysis revealed more varied mitochondrial morphology in the germlines of CX11314 compared to N2 even in the absence of defective mtDNA. We propose that asymmetric mitochondrial quality in the germline of these wild isolates results in non-uniform mtDNA inheritance, which can lead to complete removal of defective mtDNA.

104 Lineage-resolved analysis of embryonic gene expression evolution in the *Caenorhabditis* nematodes

Christopher Large[1], Rupa Khanal[2], LaDeana Hillier[3], Chau Huynh[4], Connor Kubo[4], Junhyong Kim[1], Robert Waterston[4], John I Murray[11]University of Pennsylvania, [2]Genetics, University of Pennsylvania, [3]Genome Sciences, University of Washington, [4]University of Washington

What constraints govern the evolution of gene expression patterns across development remains a fundamental question of evolutionary biology. Single-cell sequencing can help define these constraints through systematic profiling of homologous cells across different organisms. Combined with the defined and evolutionarily conserved developmental lineage of the *Caenorhabditis* nematodes, single-cell sequencing allows for the direct comparison of gene expression between homologous cell-types across evolution. Here, we have measured the spatiotemporal divergence of gene expression across embryogenesis by collecting, annotating, and comparing the transcriptomes of homologous embryonic cell types, using a dataset comprising >200,000 *C. elegans* and >190,000 *C. briggsae* cells. We find a high level of similarity in gene expression programs between the species despite tens of millions of years of evolutionary divergence. Even still, thousands of genes show divergence in their cell-type specific expression patterns, including expected categories such as G-coupled protein receptors and neuropeptides. Comparisons of transcriptome conservation between homologous cell types reveals that the neurons have diverged more than other cell types, such as the intestine, body wall muscle and germline. Across development, we find a characteristic 'hourglass' like pattern where the midpoint of embryogenesis appears under higher constraint with a lower level of divergence in gene expression between species. However, this pattern varies across major cell classes, possibly indicating function dependent constraints on developmental gene expression. We identify numerous genes with changes in the timing of expression suggesting substantial heterochronic evolution. Finally, exploring expression conservation of duplicated genes identifies numerous cases where one duplicate preferentially retains expression, a subset where both are maintained, and several with novel expression patterns. Overall, our work identifies likely constraints on the evolution of developmental gene expression and provides a powerful resource for addressing diverse evolutionary questions. To increase accessibility of these data to the community, we have generated an online visual explorer of embryonic gene expression for *C. elegans* and *C. briggsae* (https://cello.shinyapps.io/cel_cbr_embryo_single_cell/). We have collected an additional >270,000 cells across 12 *Caenorhabditis* species to further systematically identify genes and cell types that are under constraint or undergoing rapid evolution. We plan to use these data to interrogate the conservation of developmental fate regulators and explore the evolution of new cellular functions across the *Caenorhabditis* nematodes.

105 Analysis of *Caenorhabditis* tetraploids reveals the mechanisms underlying Haldane's rule

Jonathan Harbin[1], Yongquan Shen[1], Ronald E Ellis[21]Cell and Molecular Biology, Rowan-Virtua SOM, [2]Cell and Molecular Biology, Rowan Virtua SOM

In 1922 Haldane noted that when two species form hybrids, if "one sex is absent, rare, or sterile, that sex is the heterozygous sex." Haldane's rule applies to a wide variety of species, but it is still unclear what mechanisms underlie it. Since crosses between the closely related nematode species *C. nigoni* and *C. briggsae* obey Haldane's rule, we are using them to define how it works.

Woodruff *et al* observed that crosses between *C. nigoni* males and *C. briggsae* hermaphrodites produced no living males, and the reciprocal crosses between *C. briggsae* males and *C. nigoni* females produced only sterile males. By manipulating sex determination and ploidy we have shown that Haldane's Rule is due to chromosome incompatibilities (dominance theory) rather than male-specific gene evolution (faster male theory). To do this, we adapted the Schvarzstein method for producing polyploid *Caenorhabditis*. Through inactivation of *rec-8* by RNA interference we produced a *C. nigoni* tetraploid strain, the first in a male/female nematode. (A *C. briggsae* tetraploid strain already existed). DAPI staining and crosses confirmed that the *C. nigoni* females are *XXX:AAAA* tetraploids, whereas tetraploid males are *XX:AAAA*.

Remarkably, using these tetraploid strains, we observe *fertile* interspecies hybrid males from crosses using *C. nigoni* females, whereas the analogous diploid crosses make sterile males. We also see healthy interspecies hybrid males from crosses using *C. briggsae* females, whereas the analogous diploid crosses produce no surviving males at all. These results support the model that Haldane's rule in diploids nematodes is caused by incompatibility between the genes on the single *X* (which of necessity comes from only one of the two species), and interacting products made by pairs of autosomes from both species. This explanation predicts that negative interactions should be minimized in tetraploid males, which have an *X* from each parent species. Our results confirm this.

Since we recently made the first *C. nigoni* sex-determination mutations, we also tested the faster male hypothesis by a different approach. We used *tra-1* null alleles from both species to make diploid *XX* hybrids that are homozygous *tra-1(null)* mutants. These animals develop as normal males and make sperm. Thus, the problems with the *XO* hybrids are not due to incompatible male genetic programs.

Putting all these results together, the underlying cause of Haldane's Rule in *Caenorhabditis* hybrid *XO* animals is dominant incompatibilities involving the *X* chromosome, rather than the faster divergence of genes needed for male development and reproduction. Because Haldane's Rule sheds light on the origins of genetic barriers between related species, these studies also show the value of *Caenorhabtidits* in probing fundamental mechanisms of speciation.

106 A genome-wide association study of *Caenorhabditis elegans* quantitative behaviors

Maya K. Mastronardo[1], Tanara V. Peres[2], André E.X. Brown[2], Erik C. Andersen[1]1Johns Hopkins University, [2]Imperial College London

Many species vary in behaviors across populations of different individuals. *Caenorhabditis elegans* is no exception, and the molecular mechanisms of behavioral variation in response to oxygen and heat stress, foraging, egg-laying, and other behaviors have been defined. However, these studies were challenging because of the difficulties in quantifying behavior and scaling assays to analyze numerous wild strains. New high-throughput video-recording platforms and tracking technologies using Tierpsy Tracker have addressed these challenges by enabling quantitative analyses of behavioral differences, particularly crawling and postural behaviors. The goal of the Tierpsy Tracker software is to maximize the range of behavioral traits while remaining interpretable, covering the nematode's morphology, posture, path of movement, and a variety of velocities. Traits are localized to body regions, like the tip of the head. Then, time derivatives are also calculated for time-series features, such as speed of the head. Traits are categorized by movement state, such as forward, backward, or paused, and statistically summarized for each video. This approach yielded over 3,000 behavioral traits across 207 wild strains. Many traits are correlated, either due to measurement definitions or underlying biological relationships. Analyzing natural variation in these correlations and where a correlation could break can provide insights into phenotypic modularity of these natural behavioral differences. Because of the strong correlations among traits, we employed a combination of hierarchical clustering and recursive feature elimination to identify a reduced set of traits that best represent behavioral differences. To uncover the genes underlying these differences, we performed genome-wide association studies , identifying 57 traits with significant quantitative trait loci (QTL) or genomic regions correlated with differences in a behavioral trait. Further analysis focused on candidate genes within these loci. For example, a QTL on chromosome IV is associated with various radial and angular velocities across different body regions. This QTL contains a high-impact missense variant in *cec-2*, a chromodomain-encoding gene, suggesting a transcriptional role in behavioral differences. These findings highlight candidate genes as contributors to natural behavioral variation.

107 The population genomics of *Caenorhabditis tropicalis*

Bowen Wang[1], Nicolas D. Moya[1], Robyn E. Tanny[1], Michael E. G. Sauria[1], Ryan McKeown[2], Ayeh Khorshidian[1], Lewis Stevens[3], Claire Buchanan[2], Timothy A. Crombie[2,4], Clayton Dilks[2], Kathryn S. Evans[2], Daniel E. Cook[2], Gaotian Zhang[2,5], Loraina Stinson[2], Nicole Roberto[2], Daehan Lee[2,6], Stefan Zdraljevic[2], Clotilde Gimond[7], Charlie Gosse[5], Mu-En Chen[8,9,10], Viet Dai Dang[10,11], John Wang[10], Asher D. Cutter[12], Matthew V. Rockman[13], Marie-Anne Félix[5], Christian Braendle[7], Erik C. Andersen[11]Biology, Johns Hopkins University, [2]Molecular Biosciences, Northwestern University, [3]Tree of Life, Wellcome Sanger Institute, [4]Biomedical and Chemical Engineering and Sciences, Florida Institute of Technology, [5]Institut de Biologie de l'Ecole Normale Supérieure, CNRS, Inserm, [6]Biological Sciences, Sungkyunkwan University, [7]Université Côte d'Azur, CNRS, Inserm, IBV, [8]Biodiversity Program, Taiwan International Graduate Program, Academia Sinica, [9]Department of Life Science, National Taiwan Normal University, [10]Biodiversity Research Center, Academia Sinica, [11]Southern Institute of Ecology, Institute of Applied Material Science, Vietnam Academy of Science and Technology, [12]Ecology & Evolutionary Biology, University of Toronto, [13]Department of Biology and Center for Genomics & Systems Biology, New York University

Self-fertilization evolved three independent times in the *Caenorhabditis* genus, including the widely studied model organism *Caenorhabditis elegans* and the more recently discovered *Caenorhabditis tropicalis* species. This mode of reproduction influences the population genomics of species, where levels of genomic variation are typically lower than found in outcrossing species of the same genus. Lower levels of variation are predicted to reduce adaptive potential. To date, only a small number of *C. tropicalis* wild strains have been characterized for genomic variation. Broad surveys of variation across the species will enable studies of relatedness, the influence of geography and niche on species-wide variation, and signatures of selection. We obtained 809 *C. tropicalis* wild strains, organized field collection data, and performed whole-genome short-read sequencing to create a species-wide dataset. A total of 518 distinct genotypes, referred to as isotypes, were identified from seven different geographic regions around the world. The results show that, unlike *C. elegans*, the global population structure of *C. tropicalis* is strongly correlated with geography such that isotypes from Hawaii and Taiwan harbor higher genetic variation than isotypes from the Caribbean, Central America, and South America. In addition, based on the linkage disequilibrium analysis, isotypes from Taiwan could be the most ancestral population sampled to date. Moreover, the *C. tropicalis* genome has prevalent and punctuated genomic regions of extreme genetic variation, which cover approximately 31% of the NIC58 reference genome, referred to as hyper-divergent regions (HDRs). Like in *C. elegans*, these regions might offer clues into how this species is globally distributed. Interestingly, the distribution of possible toxin-antidote haplotypes, which kill offspring that do not inherit them and thereby contribute to outcrossing incompatibilities, are not strongly correlated with geography, despite the overall strong correlation of *C. tropicalis* global population structure with geography. In conclusion, this study represents the most detailed global survey of the selfing species *C. tropicalis* to date, documents that the species preserved genomic variation by maintaining HDRs within its genome and reveals strong geographic structure to its variation.

108 Signals of recent adaptive evolution in the genomes of *C. remanei* and *C. latens*

Daniel D Fusca[1], Richard Jovelin[1,2], Wei Wang[1,3], Cristel G Thomas[1], Athmaja Viswanath[1], Janna L Fierst[4,5], John H Willis[4], Patrick C Phillips[4], Asher D Cutter[11]Department of Ecology & Evolutionary Biology, University of Toronto, [2]Ontario Institute for Cancer Research, [3]Bioinformatics Resource Center, The Rockefeller University, [4]Institute of Ecology and Evolution, University of Oregon, [5]Department of Biological Sciences, Florida International University

Understanding how adaptive evolution shapes genomes is a major goal of evolutionary biology. Species with very large population sizes and high genetic diversity, such as many outcrossing *Caenorhabditis* nematode species, make for intriguing study systems to determine the factors that affect adaptive molecular evolution across different parts of genomes. Genome-wide studies of adaptive molecular evolution, however, are challenging in *Caenorhabditis* owing to high genetic divergence between most species. To address these population genetic questions, we sequenced the genomes of 35 strains of *C. remanei*, including 15 individual worms isolated directly from the wild, and 6 strains of the closely-related species *C. latens*. Demographic analyses indicate only weak differentiation between geographically separated *C. remanei* populations and declines in effective population size over time. Candidate genes potentially involved in recent selective sweeps in *C. remanei* are enriched for chemoreceptors, reminiscent of previous findings in *C. elegans*. Integrating both within-species variation and between-species divergence in a genome-wide set of genes, we find that over half of the DNA substitutions in coding sequences between *C. remanei* and *C. latens* may be adaptive, comparable to observations in mice and flies. Even against this background of prevalent positive selection, we identified groups of genes with elevated rates of adaptive evolution. For instance, we found that Argonaute genes involved in small RNA-mediated gene regulation had over twice the rate of adaptive evolution compared to the genome-wide background rate. Our results demonstrate that, despite the very strong conservation of morphology and cellular development between most *Caenorhabditis* species, their genomes accumulate extensive adaptive genetic changes even on recent evolutionary timescales. Further study of genes under positive selection in *C. remanei* and *C. latens* will help shed light on the biological processes experiencing adaptive divergence.

109 The 959 Nematode Genomes Project: the first 100 genomes

Martha Mercy Mulongo[1], Manuela R Kieninger[1], Lewis Stevens[1], Erna King[1], Pablo Gonzalez de la Rosa[1], Mark Blaxter[1,21]Tree of Life, Wellcome Sanger Institute, [2]Tree of LIfe, Wellcome Sanger Institute

The nematode Caenorhabditis elegans has been a cornerstone of biological research, providing insights into genetics, development, and neuroscience. However, C. elegans represents just one nematode species among millions. Its genome, the first metazoan genome to be fully sequenced, remains one of the few high-quality nematode genomes available. To bridge this gap, the 959 Nematode Genomes Project aims to generate chromosome-level reference genomes for hundreds of species and make them freely available to the nematode research community. For every species, we generate PacBio HiFi long-read, chromatin conformation capture (Hi-C), and short-read RNA sequencing data. For culturable species, we sequence genomes from pools of inbred individuals, while for unculturable species, including animal parasites, we can successfully generate complete genomes from single individuals.

We have now generated our first 100 chromosome-level reference genomes and of which the majority of sequenced species belong to clade V (Rhabditina), including 30 newly sequenced Caenorhabditis genomes. We have also generated genomes for species that are far more distantly related to C. elegans, including for two nematomorph outgroup species. Of the first 100 genomes, 70 come from free-living species grown in culture, with the majority of the rest representing animal parasitic species, including major livestock parasites. Notably, over a quarter of the sequenced species undergo programmed DNA elimination (PDE), a process in which portions of the somatic genome are selectively removed during development, leading to distinct germline and somatic genomes. Although most species share the haploid chromosome number of C. elegans (n=6), we observe striking variation in gene content across chromosomes within our dataset.

By vastly expanding the number of high-quality nematode genomes, our project will facilitate new research into the evolution of genome organisation, development, and key gene families across this diverse and ecologically significant phylum. Collectively, these new resources and findings will help to place C. elegans and the vast body of knowledge of its biology within a rich evolutionary context.

110 RNAi in pooled populations enables high-throughput detection of background-dependent effects

Stefan Zdraljevic[1,2], Joshua S Bloom[1,2], Leonid Kruglyak[1,21]UCLA, [2]HHMI

Each human genome encodes around 100 loss-of-function mutations, some of which cause disease. These disease-causing mutations can interact with other genetic variants to modulate disease severity among individuals. Through identification and classification of such modifier alleles, we can gain a deeper understanding of the compensatory mechanisms that render some individuals asymptomatic while others with the same mutation develop severe conditions. However, the context-dependent nature of such alleles makes them challenging to identify in a comprehensive manner.

The wild *C. elegans* population has been shown to harbor genetic variation that modifies responses to gene perturbations. These observations were made by feeding a limited number of wild *C. elegans* isolates RNAi bacteria to knock down a gene of interest and quantifying the response of each isolate. We sought to build on these results by testing the effects of genetic backgrounds on RNAi-induced perturbations at high throughput. To this end, we developed an experimental approach and computational tools to infer the frequencies of hundreds of individual *C. elegans* isolates grown in pooled populations from low-coverage WGS data. This approach enables us to accurately and efficiently assess the responses of isolates to gene perturbations by quantifying their frequencies before and after exposure to RNAi bacteria. We can accurately infer the frequencies of 100 isolates with as little as 1X sequencing depth, and of up to 550 isolates with sequencing depth of 10X.

We used this pooled-phenotyping approach to assess the germline *pos-1* RNAi response of 370 *C. elegans* isolates in a single flask. After classifying isolates as germline RNAi responders and non-responders, we validated five distinct loci that modulate germline RNAi efficacy. We resolved one locus to ~400 kb using NILs, and further fine-mapping is in progress. In addition to measuring germline RNAi efficacy across the population, the *pos-1* RNAi phenotyping allowed us to select and pool isolates sensitive to germline RNAi. We used this pooled population to measure the isolate-specific effects of RNAi knockdown of several genes that are known to have large knockdown effects in N2. In agreement with previous reports, we discovered multiple large-effect loci that modify responses to RNAi-induced perturbations. We are now poised to greatly expand the scope of exploring background-dependent effects of RNAi perturbations across hundreds of RNAi-susceptible *C. elegans* isolates.

111 SRD-12: A novel olfactory GPCR for regulating foraging in *C. elegans*

Ritika Siddiqui[1], Nikita Mehta[1], Gopika Ranjith[2], Marie-Anne Félix[3], Changchun Chen[4], Varsha Singh[51]Molecular Microbiology, University of Dundee, [2]Max Planck Institute, [3]Institute of Biology of the Ecole Normale Supérieure (IBENS), [4]Umeå University, [5]University of Dundee

Acquisition of essential nutrients through diet is crucial for the survival of animals. Dietary odors might enable foraging in animals for nutrient-rich diets. We asked if *Caenorhabditis elegans*, a bacterivorous nematode, uses olfactory cues to forage for essential amino acid-rich (EAA) diets. Using the native microbiome of *C. elegans,* we show that worms rely on olfaction to select leucine (EAA)-enriched bacteria. Using gas chromatography, we find that leucine-enriched bacteria produce isoamyl alcohol (IAA) odor in the highest abundance. Prior adaptation of worms to IAA diminishes the diet preference of worms. Several wild isolates of *C. elegans* display robust responses to IAA emphasizing its ecological relevance. We find that foraging for a leucine-enriched diet is mediated via the AWC olfactory neurons. Finally, we identify SRD-12, in AWC neurons, as a receptor for IAA and a mediator of dietary decisions in worms. Our study identifies a receptor-ligand module underpinning foraging behavior in *C. elegans*.

112 *Caenorhabditis* nematodes self-assemble into living towers for collective dispersal

Daniela M. Perez[1,2], Ryan Greenway[1], Thomas Stier[1,3], Narcís Font Massot[1,2], Serena Ding[1,21]Max Planck Institute of Animal Behavior, [2]Centre for the Advanced Study of Collective Behaviour, [3]Study program at Department of Biology, University of Konstanz

Dispersal behavior allows organisms to find new resources under harsh conditions; collective dispersal in group-living organisms raises interesting questions about kin selection, cooperation, and social conflicts that offer an exciting window into the evolution of sociality. One type of collective dispersal is when individuals physically link their bodies into a super-organism and move as a group, like in some nematode species where individuals self-assemble into a living tower. However, direct evidence demonstrating the occurrence and the function of nematode towers in nature has been scarce. We documented disturbance-free behavior of 52 towers directly in the wild to confirm their existence, and manipulated them to show that they can bridge gaps and confer group dispersal by phoresy. Using a new laboratory assay with the model organism *Caenorhabditis elegans*, we also revealed the effect of life stages and strain sociality on towering behavior. Interestingly, we found that worms from all life stages can tower in the lab, even though the towers we found in nature consist exclusively of dauers. Furthermore, worms from different positions in the tower do not differ in their reproductive fitness, suggesting that the behavior is potentially cooperative in this species. Finally, we found natural variation in towering behavior in a set of 12 *C. elegans* wild isolates, and show that this behavioral variation likely has a genetic component. Our work sets the key foundations to establish nematode towering behavior as a powerful opportunity to elucidate the ecology, the mechanisms, and the evolution of collective dispersal.

113 Identification of a potential mucin required for bacterial adherence to the intestinal epithelium of *C. elegans*

Cayla Cruse, Dalaena Rivera, Anupama Singh, Robert Luallen San Diego State University

Wild *Caenorhabditis* nematodes are naturally infected and colonized with a diverse array of microbes that comprise its gut microbiome. Through ecological sampling, we have identified a bacterial species that directionally binds to the intestinal epithelial cells of several *Caenorhabditis* isolates, including *C. elegans*. This adherent bacteria, *Lelliottia jeotgali* (LUAb3), can replicate in the gut and has a neutral effect on host fitness suggesting it is a commensal microbiome bacteria.

To identify host factors that are required for LUAb3 adherence, we conducted a forward genetic screen using a red fluorescent LUAb3 strain and identified 40 mutants that are colonization deficient. We conducted in silico complementation and found 5 genes with multiple alleles, which we named them *kola-1-5,* for knockout of ***L****elliottia* adherence. We conducted complementation of the *kola* mutants and validated that these gene are required for adherence. We did protein and structural analysis of KOLA-1 and identified that it is likely a membrane-bound mucin, with a highly O-linked glycosylated PTS domain and a distal globular domain. Interestingly, all of our mutations were in the distal globular domain, suggesting that this domain is required for bacterial adherence. When we disrupted O-linked glycosylation with a *gly-5* mutant, we were able to see a decrease in LUAb3 colonization. Additionally, KOLA-1 has a low isolectric point, in line with the pH of the gut lumen. We were able to disrupt LUAb3 blinding by increasing the lumenal pH chemically and genetically.

In conclusion, we have identified a putative receptor for bacterial adhesion, the membrane bound mucin KOLA-1. The findings from this research could improve our understanding of host adhesion by microbiome bacteria and inform future studies in host-microbe interactions.

114 From cytosol to centrosome: the evolution of novelty through the lens of a selfish element

Julian Ross[1], Polina Tikanova[1], Andreas Hagmüller[1], Maximilian Spicer[1], Valeria Stefania[1], Manuel Hunold[1], Danny Nedialkova[2], Alejandro Burga[11]Institute of Molecular Biotechnology (IMBA), [2]Max Planck Institute for Biochemistry

Gene duplication is a fundamental evolutionary mechanism by which protein families expand and diversify. However, the extent to which duplicated genes acquire entirely novel molecular functions—beyond subtle modifications of existing ones—remains poorly understood, especially at the molecular level. To address this gap, we investigated how FARS-3—an essential subunit of the phenylalanyl-tRNA synthetase complex—rapidly evolved into KLMT-1, a potent toxin that is part of a selfish toxin-antidote (TA) element in *C. tropicalis*.

Surprisingly, we found that despite originating from a tRNA synthetase, KLMT-1 does not kill embryos by disrupting the abundance or charging of Phe-tRNA (or any other tRNA). Instead, we discovered that KLMT-1, unlike its essential ancestor FARS-3, localizes to the centrosome of developing embryos. Remarkably, this localization, as evidenced using centrosomal markers, is not constitutive but specifically triggered during early embryogenesis. We identified key amino acid differences between KLMT-1 and FARS-3, including a 3-amino acid insertion in an otherwise highly similar region, that are required for both centrosomal localization and toxicity. Preliminary evidence further suggests that KLMT-1 interacts with AIR-1 (Aurora kinase A), a major mitotic regulator, and that this interaction is essential for its localization and toxic function. In line with these findings, our preliminary results suggest that KLMT-1 expression in HeLa cells induces cell division abnormalities, consistent with a role as a disruptor of centrosome function.

Collectively, our results illustrate how radically new molecular functions can arise through gene duplication and highlight how selfish TAs can serve as powerful platforms for organisms to explore otherwise inaccessible sequence space of essential genes—potentially shaping long-term evolutionary trajectories of the host beyond the lifespan of the selfish element itself.

115 Reconstituting the force transmission pathway during touch – revisiting the role of stomatin-microtubule interaction

Neus Sanfeliu-Cerdán, Martín Fernández-Campo, Michael Kriegl CFO - The Institute of Photonic Sciences

Mechanical forces propagate through tissues and are converted into physiological signals – a process known as mechanotransduction. While mechanosensitive ion channels are well described, the mechanisms by which forces reach these molecular sensors remain unclear. Here, we revisit the role of microtubules in the force transmission pathway during touch.

C. elegans perceives gentle mechanical forces to their body wall with their six touch receptor neurons (TRNs). MEC-4, the pore-forming subunit of the mechanosensitive ion channel interacts with MEC-2, a conserved protein with structural similarities to Stomatin. However, the biophysical properties and mechanistic role of MEC-2 in force transmission remain poorly understood. Recently, we found that MEC-2 forms biomolecular condensates through liquid-liquid phase separation. These condensates undergo a viscoelastic phase transition and exhibit a frequency-dependent response to mechanical stress - they transmit forces at high frequencies (>1 Hz) but not at lower ones, suggesting a mechanistic role in tuning the frequency response of the worm to external touch.

More than 3 decades ago, it was shown that MEC-12 α-tubulin genetically interacts with the MEC-2 domain responsible for viscoelastic phase transitions. We further show that MEC-2 condensates co-assemble and host α/β-tubulin. We found that this interaction modulates the mechanical properties of the condensates, providing the first indication of how microtubules may participate in the force transmission pathway in touch sensation.

This work establishes a novel physiological role for biomolecular condensates, demonstrating that their maturation state fine-tunes neuronal mechanotransduction and offers a broader conceptual framework for understanding how animals perceive and respond to mechanical forces.

116 Programmed DNA elimination was likely present in the last common ancestor of *Caenorhabditis* nematodes

Lewis Stevens[1], Simo Sun[2], Nami Haruta[3], Leyun Xiao[2], Naoki Uwatoko[3], Manuela Kieninger[1], Kazuki Sato[2], Akemi Yoshida[2], Dominic Absolon[1], Joanna Collins[1], Asako Sugimoto[3], Taisei Kikuchi[2], Mark Blaxter[11]Wellcome Sanger Institute, [2]The University of Tokyo, [3]Tohoku University

In most organisms, including *C. elegans*, all cells inherit the same genome, and many mechanisms exist to preserve its integrity across cell divisions. In contrast, some species undergo programmed DNA elimination (PDE), a process in which specific regions of the genome are selectively removed in the somatic lineages early during development, resulting in distinct germline and somatic genomes. Since its discovery over a century ago in parasitic nematodes, PDE has been observed in diverse eukaryotic lineages, including ciliates and vertebrates. However, its function, mechanisms, and evolutionary origins remain poorly understood. Here, we report our unexpected discovery of PDE in three early-diverging *Caenorhabditis* species. We found that multiple genomic regions - including all telomeres - are eliminated from somatic blastomeres at the 8-16-cell stage, while the germline genome remains intact. This elimination fragments the six germline chromosomes into between 8 and 15 somatic chromosomes. We used FISH and live imaging of GFP::Histone to demonstrate that telomere shortening and DNA cleavage occur before metaphase. Following nuclear envelope breakdown, the eliminated DNA fragments migrate toward the cell cortex without associating with spindle microtubules, leading to their exclusion from the nucleus. We used long-read and Hi-C genome sequencing data to reconstruct the germline and somatic genomes and found that between 0.7 and 2.3% of the germline genome is eliminated in the three species. We identified a short motif at the elimination sites - which we term the chromosome break sequence (CBS) - that likely recruits an enzyme to induce double-strand breaks, which are subsequently healed by de novo telomere addition. The eliminated DNA contains several genes that are essential for *C. elegans* germline function, including multiple RNA-binding proteins (such as *nos-1*, *rnp-8*, and *puf-8*) that regulate the translation of germline mRNAs. We also found that elimination sites coincide with sites of genome rearrangement, suggesting that PDE has influenced the evolution of genome organisation in *Caenorhabditis*. By reconstructing the evolutionary history of PDE, we show that it was likely present in the last common ancestor of *Caenorhabditis* but was lost early during the evolution of *C. elegans* and related species. Our findings challenge the view of PDE as a rare, taxonomically-restricted phenomenon and instead suggest that PDE was a key developmental process in early *Caenorhabditis* evolution that had an important influence on genome organisation and regulation.

117 Proteolytic cleavage of synaptic adhesion molecules regulates aversive learning in *Caenorhabditis elegans*

Yu-Hsuan Liu[1,2], Yen-Ju Chen[2,3], Chun-Liang Pan[1,21]Institute of Molecular medicine, College of Medicine, National Taiwan University, [2]Center for Precision Medicine, College of Medicine, National Taiwan University, [3]Institute of Molecular Medicine, College of Medicine, National Taiwan University

Aversive learning enables protective avoidance behavior that minimizes exposure to predators or environmental threats and is crucial for the fitness and survival of the animals. The nematode *Caenorhabditis elegans* undergoes aversive learning for bacterial cues under concomitant mitochondrial stress. We find that the synaptic adhesion molecule CASY-1/calsyntenin promotes stress-induced aversive learning through proteolytic cleavage of its N-terminal ectodomain, echoing prior studies that show CASY-1 cleavage occurs during salt learning. Sensory input during stress exposure increases CASY-1 cleavage, and increased CASY-1 cleavage during stress is blocked by acute inhibition of head sensory neurons, highlighting the importance of these neurons in CASY-1 processing. We further discovered that the α-secretase *sup-17* and components of the γ-secretase complexes, including the presenilins *hop-1* and *sel-12*, and *aph-1*, are essential for aversive learning, and all of them promote CASY-1 shedding under stress. Genetically, CASY-1 likely acts through BAM-2, a neurexin-like transmembrane receptor, with LIN-2/CASK, LIN-7/Veli, and LIN-10/Mint1 functioning in the same pathway as *bam-2*. These findings provide insights into the molecular mechanisms by which proteolytic cleavage of synaptic adhesion molecules regulates stress-induced aversive learning in *C. elegans*. (Supported by National Science and Technology Council NSTC-113-2320-B-002-024-MY3)

118 Molecular patterns of evolutionary changes throughout the whole nervous system of multiple nematode species

Itai Antoine Toker[1], Lidia Ripoll-Sánchez[2], Luke T Geiger[1], Karan S Saini[1], Isabel Beets[3], Petra E Vértes[4], William R Schafer[2,3], Eyal Ben-David[5], Oliver Hobert[6]1Biological Sciences, Columbia University, [2]MRC-LMB, [3]KU Leuven, [4]University of Cambridge, [5]Hebrew University of Jerusalem, [6]HHMI, Columbia University

One avenue to better understand brain evolution is to map molecular patterns of evolutionary changes in neuronal cell types across entire nervous systems of distantly related species. We generated whole-animal single-cell transcriptomes from L2 larvae of all known hermaphroditic *Caenorhabditis* species (*C.elegans, C.briggsae* & *C.tropicalis*), and complemented the datasets with multiple CRISPR-inserted endogenous reporter alleles that labelled genes acting in their nervous systems. We observed a remarkable stability of neuronal cell type identities and found that conserved patterns of combinatorial expression of homeodomain transcription factors are among the best classifiers of homologous neuron classes. Unexpectedly, we discover an extensive divergence in neuronal signaling pathways. While the identity of the particular neurotransmitters deployed in homologous neurons remains stable (glutamate, acetylcholine, GABA, different monoamines), expression of ionotropic and metabotropic receptors for all these neurotransmitter systems shows substantial divergence, resulting in more than half of all neuron classes changing their capacity to be receptive to specific neurotransmitters. Neuropeptidergic signaling is also exceptionally divergent, both at the level of neuropeptide expression and receptor expression, yet the overall dense network topology of the wireless neuropeptidergic connectome remains conserved. Notably, pharyngeal neurons were disproportionally enriched for incoming and outgoing species-specific connections, highlighting the enteric nervous system as a potential hotspot for rapid evolutionary novelty in its peptidergic connectivity. Finally, we characterize a new family of small secreted proteins that show no obvious hallmarks of conventional neuropeptides, but show similar patterns of highly neuron-type-specific and highly evolvable expression profiles, perhaps hinting to yet unappreciated axes of neuronal signaling in worms. In summary, by investigating the evolution of entire nervous systems at the resolution of single neuron classes, we uncover patterns that may reflect basic principles governing evolutionary novelty in neuronal circuits.

119 *C. elegans* avoids *Todstoff*, a novel necrotaxis cue from dead worms

Erik Toraason[1], Rachel Kaletsky[1], Alden Weiner[2], Waasae Ayyaz[2], Mirian Rodriguez[2], Xuanjia Yu[3], Tom W Muir[3], Coleen T Murphy[11]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [2]Molecular Biology, Princeton University, [3]Chemistry, Princeton University

To survive in hazardous environments, organisms must navigate and avoid myriad threats, including those that might have already killed their kin. Many species across diverse phyla detect warning cues in the remains of dead or injured conspecifics, allowing them to enact defensive strategies to avoid a similar fate. We have discovered that *Caenorhabditis elegans* senses a novel aversive cue, 'Todstoff' ("death stuff"), that is present in the remains of dead worms and induces living conspecifics to perform negative necrotaxis behaviors. *Todstoff* is distinct from known social signals in *C. elegans*, including ascaroside and alarm pheromones, and can be biochemically isolated from worm lysate. We determined that *Todstoff* is sensed by the ASH polymodal nociceptive neurons, which mediate necrotaxis via GPCR and synaptic glutamatergic signaling. Furthermore, we found that *C. elegans* integrates social pheromone, food quality, and necrotaxis cues to inform their behavioral decisions. Via biochemical fractionation, chemical spectrometry, forward genetics, and comparative biology, we are uncovering the identity of *Todstoff* and molecular pathways that mediate this behavior. Taken together, our work illuminates a post-mortem inter-animal signaling pathway that promotes death avoidance.

120 Dopamine signaling drives skin penetration by mammalian-parasitic nematodes

Ruhi Patel, Elissa A Hallem University of California, Los Angeles

Skin-penetrating nematodes, including *Strongyloides stercoralis* and hookworms in the genera *Necator* and *Ancylostoma,* infect over 8% of the global population, causing debilitating disease and fatalities. Skin penetration, the process whereby these nematodes invade host skin and enter the body, is a critical but poorly understood step of the infection process. We are studying skin-penetration behavior using the human-parasitic nematode *S. stercoralis*.

To examine skin penetration, we established an *ex vivo* assay wherein infective third-stage larvae (iL3s) are placed on excised rat skin and video-recorded. We found that iL3s pushed down on the skin with their heads almost immediately following contact. Thereafter, iL3s either initiated penetration by puncturing the skin with their heads or crawled a short distance on the skin. Some punctures led to complete penetration whereas others were aborted. Ultimately, iL3s repeatedly pushed, punctured, and crawled on skin until penetration was completed. Furthermore, we observed more pushes and punctures on textured skin surfaces, relative to smooth surfaces, indicating that skin surface texture modulates skin-penetration behavior.

We next investigated the neural mechanisms that underlie skin penetration and hypothesized that the texture-sensing neurons might be necessary. *C. elegans* senses texture using the dopaminergic neurons. Thus, as an initial test of our hypothesis, we treated iL3s with the dopamine receptor antagonist haloperidol and did skin penetration assays. We found that haloperidol inhibited skin penetration in both *S. stercoralis* and the human hookworm *Ancylostoma ceylanicum*; these defects were rescued upon the addition of exogenous dopamine. Thus, dopamine signaling likely plays a conserved role in driving skin penetration in multiple species of skin-penetrating parasitic nematodes. Inhibition of skin penetration was also observed upon CRISPR/Cas9-mediated genetic inactivation of dopamine signaling and chemogenetic silencing of the dopaminergic neurons in *S. stercoralis*. Additionally, inactivation of the TRPN channel *Ss*-TRP-4 nearly eliminated penetration of both rat and human skin by *S. stercoralis*. Together, our results provide mechanistic insight into how skin-penetrating nematodes invade hosts and suggest that drugs targeting TRP-4 or other nematode-specific components of the dopaminergic pathway could be developed into topical prophylactics that block skin penetration, thereby preventing infections.

121 A variant ionotropic receptor expressed exclusive in the I3 pharyngeal enteric neuron senses salts to regulate high salt stress

Jihye Yeon, Laurie Chen, Nikhila Krishnan, Sam Bates, Charmi Porwal, Piali Sengupta Biology, Brandeis University

Gut-intrinsic enteric neurons detect chemicals present in the gut lumen to broadly regulate behavior and physiology. However, the molecules and pathways that underlie interoceptive chemosensation are poorly described. The *C. elegans* pharyngeal neuronal network is anatomically and functionally homologous to the mammalian enteric nervous system; we refer to these neurons as pharyngeal enteric neurons (PENs). While 15 out of 20 PENs exhibit sensory-motor characteristics (1), only the I2 and NSM neurons have been shown to have sensory functions (2,3). Variant ionotropic receptors (IRs) are present in all protostomes. However, to date, chemosensory functions for these proteins have been established only in insects. Here, we show that the IR-related protein GLR-9 in *C. elegans* is localized to the tip of the I3 PEN, where it is directly exposed to the pharyngeal lumen. GLR-9 trafficking and localization requires GLR-7, the homolog of the IR25a co-receptor. We find that GLR-9 is necessary for responses to a broad range of monovalent and divalent cations in I3, and misexpression of GLR-9 and GLR-7 in the non-salt-sensing I1 PENs is sufficient to confer salt responses. We further show that GLR-9-mediated salt detection in I3 is crucial for survival specifically on high salt but not on high sugars with or without prior acclimation. Cholinergic signaling from I3 protects against acute high salt stress, whereas peptidergic signaling from I3 via the FLP-6 neuropeptide is essential for survival on high salt following acclimation. Transcriptomic analysis reveals that salt acclimation alters expression of genes implicated in the regulation of metabolism and stress, as well as cuticular remodeling. This altered gene expression program requires both GLR-9-dependent and independent pathways. Taken together, our results identify a chemosensory function for a variant IR beyond insects, and highlight the critical function of a single enteric neuron in regulating physiological homeostasis under high salt stress.

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122 Working Memory in *C. elegans* emerges from internalized distributed motor command oscillators

Raymond L Dunn[1], Caitriona M Costello[2], Jackson M Borchardt[1], Daniel Y Sprague[1], Grace C Chiu[1], Julia M Miller[3], Noelle D L'Etoile[3], Saul S Kato[11]Neurology, University of California, San Francisco, [2]Psychiatry, University of California, San Francisco, [3]Cell and Tissue Biology, University of California, San Francisco

Any animal navigating a complex environment stands to benefit from the ability to quickly form impressions of the world, retain them internally, and act on them later. But how did nervous systems acquire this foundational memory ability? Here, we report the existence of a working memory system in *C. elegans* that is employed for deferred action in a sensory-guided decision-making process.

During foraging worms make reversal-then-turn reorientation maneuvers, in either the dorsal or ventral direction, traditionally considered an unregulated random process (Pierce, 1999). However, fast olfactory neuron signaling is necessary for chemotaxis (Kato et al. 2014), suggesting worms may integrate sensory information with body posture to navigate effectively.

We constructed a closed-loop virtual olfactory environment, combining a red-shifted depolarizing opsin, patterned illumination with a micro-mirror device, and real-time detection of head curvature (from crawling worms) or neural activity (during whole-brain imaging) to trigger optogenetic activation of the sensory neuron AWA during forward crawling, mimicking a temporally controlled asymmetric odor signal experience.

Animals showed a preference for resolving reversals with a turn in the favored direction, indicating (a) worms integrate olfactory sensation with proprioception or motor commands for headswings, and (b) to act appropriately after the reversal, the animal needs some form of working memory.

Previous studies have shown that the worm brain cycles through broadly distributed, coordinated patterns of activity corresponding to major motor behavioral states; forward/reverse crawling and turning (Kato et al. 2015). We expand on this, finding that whole-brain dynamics decompose into two distributed oscillators, one for motor command state (i.e. forward/reverse) and another for head curvature (i.e. dorsal/ventral). We find that this working memory is implemented by the interaction of the command and headswing oscillators; AWA stimulation aligns their relative phase, which persists for 30-90s and through reversal, until producing a directed turn.

We propose that the original function of these oscillators was to produce physical movement, and they later evolved to support the function of working memory, endowing animals with the ability to perform deferred sensory-guided action selection. This evolutionary step may represent the origin of a functional primitive of cognition.

123 Why Deprivation Makes Food Taste Better?

Maria Gabriela Blanco[1,2], Jeremy Florman[3], Ailin Lacour[1], María José De Rosa[1], Mark Alkema[3], Diego Rayes[11]Instituto de Investigaciones Bioquímicas de Bahía Blanca (CONICET)-Dtto. Biologia, Bioquímica y Farmacia (UNS), [2]Instituto de Investigaciones Bioquimicas de Bahia Blanca (CONICET), [3]Neurobiology, UMASS Chan Medical School

The phenomenon of food tasting better after deprivation is conserved across many species. In *C. elegans*, an enhanced slowing response is observed when encountering food after 30 minutes of food deprivation (Sawin et al., 2000). This response is mediated by a large increase in serotonin (5-HT) release from the NSM and ADF neurons (Iwanir et al., 2016; Rhoades et al, 2019). We found that this slowing response and the activity of serotonergic neurons upon food exposure are further enhanced in tyramine (TA)-deficient mutants.

TA levels decrease during fasting, and *C. elegans* reduces its locomotion rate after prolonged fasting (>30 minutes). This reduction in locomotion can be counteracted by the addition of exogenous TA, highlighting the role of TA in regulating movement. In contrast, serotonin (5-HT) decreases locomotion, indicating an antagonistic relationship between 5-HT and TA. We found that tyramine exerts an inhibitory effect on the NSM neuron by activating the tyramine GPCR receptors SER-2 and TYRA-2 Downregulation of tyramine during food deprivation leads to the disinhibition of serotonergic signaling and increased NSM activity upon re-encountering food. This disinhibition provides molecular and neural insights into the enhanced slowing response, enabling the worm to efficiently exploit a new food source. The antagonistic crosstalk between TA and 5-HT illustrates how the nervous system controls discrete behavioral state transitions during foraging. This mechanism may explain why food is perceived as more rewarding or appealing in states of hunger, offering a neural basis for the enhanced sensory experience of food after deprivation.

124 Modeling antagonistic sensors and directional confidence in *C. elegans* chemotaxis

Xuan Wan[1], Tingtao Zhou[2], Paul W. Sternbegr[31]BBE, Caltech, [2]CCE, California institute of technolog, [3]BBE, California institute of technolog

Navigating complex chemical gradients requires dynamic integration of sensory cues and adaptive behavioral strategies. We have been studying this problem the male chemotaxis in response to volatile sex pheromones, a process mediated by the SRD-1 receptor and detected through coordinated activity of both AWA and PHD sensory neurons. Here, we present a computational model that dissects how functional antagonism in *C. elegans*' dual sensory neurons and directional confidence drive precision chemotaxis. The model reveals that antagonistic interactions between AWA and PHD—through differential weighting of inputs, distinct concentration optima, and unique gradient feature extraction—generate a dynamic "directional confidence" signal. This signal governs turning direction and orthokinetic speed regulation: high confidence (suppressed PHD activity at elevated pheromone concentrations) triggers rapid acceleration (sprints) toward the source, while low confidence (active PHD at low concentrations) prevents erroneous turns in volatile environments.

We systematically integrated experimental data from whole-brain imaging, optogenetics, and microfluidics to both parameterize and validate the model. These experiments demonstrated that PHD inactivation disrupts acceleration by affecting directional confidence, while fluctuations in signal-noise ratio modulate interneuron activity to adjust decisiveness. Targeted optogenetic manipulation of target neurons further confirmed the model's prediction that behavioral plasticity arises from context-dependent integration of sensory and noise signals. A minimal-parameter computational framework recapitulates the interplay between head (AWA) and tail (PHD) signals, offering predictive power to decode adaptive navigation in shallow or noisy gradients.

By unifying experimental and modeling approaches, this work establishes how functional neuron diversity and confidence-driven decisions optimize chemotaxis. The model's simplicity and generalizability advance theoretical understanding of neural circuit computation and provide a blueprint for bio-inspired robotics to navigate complex environments with minimal computational resources.

125 Cold and Lithium delay forgetting of olfactory memories in *C. elegans*

Dana Landschaft-Berliner[1], Kesem Goldstein[2], Guy Teichman[3], Sarit Anava[3], Hila Gingold[3], Itai Rieger[3], Noam Levy[4], Yehuda Salzberg[5], Vladyslava Pechuk[5], Priti Agarwal[6], Dror Sagi[3], Dror Cohen[4], Evelina Nikelshparg[7], Anat Ben-Zvi[7], Antonio Miranda Vizuete[8], Ronen Zaidel-Bar[6], Meital Oren-Suissa[5], Oded Rechavi[31]neurobiololgy, Tel Aviv university, [2]Neurobiology, Tel Aviv university, [3]neurobiology, Tel Aviv university, [4]Tel Aviv university, [5]Brain Sciences, Weizmann Institute of Science, [6]Cell and Developmental Biology, Tel Aviv university, [7]Biotechnology, Ben-Gurion University of the Negev, [8]Redox Homeostasis, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla

Forgetting is commonly regarded as an unavoidable process, yet it can be actively regulated by physiological and molecular mechanisms. In *Caenorhabditis elegans,* we found that cold exposure significantly prolongs memory retention. This effect is abolished in worms that have adapted to low temperatures. To examine this phenomenon, we performed RNA sequencing, mutant analyses, and pharmacological assays. Our findings indicate that shifts in membrane properties control the activation and deactivation of cold induced delayed forgetting. We identified the diacylglycerol (DAG) pathway as a key regulator of forgetting and found that AWC sensory neurons are critical sites for this regulation. Additionally, lithium, a drug widely used to treat bipolar disorder, extends memory retention in a manner similar to cold exposure, but not in worms that have adapted to low temperatures. Neuronal activity recordings suggest a role for AIY interneurons in the memory trace, indicating they may contribute to memory preservation. The genetic tractability of *C. elegans* provides a powerful framework for dissecting the mechanisms underlying memory retention in response to lithium and cold, offering broader insights into how memory is stored and lost. I will present new and unpublished results shedding light on the underlying mechanism.

126 Hierarchical Competing Inhibition Circuits Govern Motor Stability in *C. elegans*

Yongning Zhang, Yunzhu Shi, Kanghua Zeng, Lili Lili Chen, Shangbang Gao Huazhong Univ. of Sci. & Tech.

Stable movement and efficient motor transition are both crucial for animals to navigate their environments, yet the neural principles underlying these abilities are not fully understood. In free-moving *Caenorhabditis elegans*, sustained forward locomotion is occasionally interrupted by backward movements, which are believed to result from reciprocal inhibition between the interneurons AVB and AVA. Here, we discovered that hierarchical competing inhibition circuits stabilize spontaneous movement and ensure motor transition. Through behavioral analysis, optogenetics, and in situ electrophysiology, we found that the modulatory interneuron PVP activated AVB to maintain forward locomotion while inhibiting AVA to prevent backward movement. Another interneuron, DVC activates AVA and forms a disinhibition circuit that inhibits PVP, thereby relieving PVP's inhibition of AVA and facilitating backward movement. Notably, these asymmetrical circuit motifs create a higher-order competing inhibition that likely sharpens the motor transition. We also identified cholinergic and glutamatergic synaptic mechanisms underlying these circuits. This study elucidates a key neural principle that controls motor stability in *C. elegans*.

127 The molecular coding of intercellular Ca2+ waves of the defecation motor program

Rebekka H Anderson, Jeremy T Florman, Mark J Alkema Neurobiology, UMass Chan Medical School

Rhythmic intercellular Ca2+ waves (ICWs) coordinate a range of important biological processes, from muscle contraction to embryonic development. ICWs are initiated by the release of Ca2+ from intracellular stores, which then propagate to neighboring cells through gap junctions. Although several proteins that modulate ICW propagation have been characterized, questions remain about how ICWs rhythmicity is regulated. To better understand the mechanisms regulating ICWs, we focused on the *C. elegans* defecation motor program (DMP), a three-step motor program driven by Ca2+ waves that propagate through the intestine every 45 seconds. To study ICWs in freely moving animals, we generated a strain expressing GCaMP6 in the intestine and developed an analysis pipeline that allows detailed quantification of Ca2+ dynamics. Using Ca2+ imaging and mutant analysis, we characterized proteins involved in Ca2+ signaling and homeostasis. *sca-1*/SERCA mutants exhibited prolonged Ca2+ peaks. *itr-1/IP3R* mutants had longer intervals while *cmd-1*/Calmodulin mutants displayed fast cycling Ca2+ waves. Mutations in the gap junction protein INX-16 cause defects in wave propagation. To uncover new genes involved in ICW rhythm, we turned to mutants with altered defecation cycles (*dec* mutants) that were identified in a previous screen1. The *dec* mutant in our GCaMP6 strain display changes only in their interval, consistent with DMP defects. Using whole genome sequencing and mapping data, we have determined the causative mutation for multiple *dec* mutants. We will present the molecular characterization of fast cyclers *dec-9* and *dec-10,* and slow cycler *dec-11*. These studies should allow us to understand how intestinal ICWs are regulated.

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128 Fertility reversibly modulates *C. elegans* behavior via gonad to nervous system signaling

Emily A Bayer Biozentrum, University of Basel

While the external cues that generate behavioral responses have been extensively studied, behavioral changes in response to internal stimuli are much less understood. *C. elegans* hermaphrodites are self-fertile until they exhaust their supply of self-sperm, after which time they can only produce more progeny via mating with a male animal. When still fertile with either self or cross sperm, hermaphrodites will evade male mating attempts.

I have found that hermaphrodite escape is facilitated by an aversive response to physical contact (touch stimulus) by males. Mating in *C. elegans* is accomplished via a stereotyped series of behavioral maneuvers by the male, and I have specifically found that fertile hermaphrodites attempt to escape from male turns around the nose, and male contact at the vulva. In contrast to this difference in response to male contact, both self-fertile and sperm-depleted hermaphrodites respond in a standard nose touch assay. This suggests that male mating touch represents a specific sensory modality.

Using inducible germline masculinizing and feminizing mutants, I have found that sperm are both necessary and sufficient to induce mating evasion. In turn, monoaminergic signaling from the somatic gonad is required to relay sperm status to the nervous system. In the absence of the HSN neuron, hermaphrodites fail to perform both the vulva evasion and nose evasion behaviors. Sensory neurons in the head are required for nose evasion, but do not feed back onto vulva evasion.

I hypothesize that HSN receives information regarding fertility status via the somatic gonad, and in turn controls vulva evasion behavior while also relaying this information to nose touch escape circuits. This paradigm highlights how animals sense fertility status to make a behavioral choice.

129 Context-dependent serotonin signaling links dietary quality to foraging decisions

Likui Feng, Audrey Harnagel, Javier Marquina-Solis, Lishu Yue, Yarden Greenfeld, Cornelia Bargmann Lulu and Anthony Wang Laboratory of Neural Circuits and Behavior, The Rockefeller University

Animals sense their metabolic needs to guide adaptive foraging decisions, partly through serotonin, a neurotransmitter associated with feeding in many species. However, whether the serotonin system encodes the capacity to interpret diverse dietary qualities remains unclear. Using the nematode *Caenorhabditis elegans*, we conducted a systematic behavioral screen of 3,983 *E. coli* knockout strains and identified 22 metabolic mutants that induce altered foraging behaviors. These "mediocre" diets, which include strains affecting bacterial metabolism, cysteine and vitamin B6 biosynthesis, ferric iron transport, and membrane integrity, are viable and support fertility but elicit distinct stress responses in *C. elegans*. Animals exposed to these diets gradually shift their foraging preferences, spending more time exploring food-free environments to seek better conditions. This behavior is distinct from responses to high-quality or pathogenic foods, highlighting *C. elegans*' nuanced ability to discriminate dietary quality.

Mechanistically, we found that the behavioral response to mediocre food is mediated by serotonin-producing NSM neurons in the enteric nervous system, which are also known to promote feeding on high-quality food. NSM neurons respond to two classes of mediocre diets: those affecting bacterial cell size/shape and inducing mitochondrial stress, and those inducing oxidative stress due to metabolic imbalance. Remarkably, NSM neurons signal through distinct serotonin receptors depending on the type of mediocre food. Serotonin receptors in the enteric nervous system mediate responses to bacterial size/shape, while receptors on neurons outside this system regulate responses to oxidative stress. In contrast, the behavioral responses to high-quality or pathogenic food is mediated by other serotonergic neurons and circuits, revealing a distributed serotonergic network that integrates food quality signals.

Our findings demonstrate that serotonin enables *C. elegans* to discriminate between high-quality, mediocre, and pathogenic diets, with each context recruiting distinct serotonergic neurons, receptors, and downstream circuits. This work underscores the versatility of serotonin in modulating foraging behaviors and reveals the logic of a neuromodulatory network that dynamically responds to dietary quality, balancing exploration and exploitation to optimize metabolic outcomes.

130 Identification of a kin-recognition receptor in *Pristionchus pacificus*

Fumie Hiramatsu, James W Lightfoot Max Planck Institute for Neurobiology of Behavior – caesar

Kin-recognition is found across diverse species and mediates an array of different behavioural interactions. These include complex organismal behaviours ranging from collective dynamics to mate preference although crucially, the molecular mechanisms behind such abilities are often unclear. In the predatory nematode *Pristionchus pacificus,* a contact-mediated kin-recognition system allows them to differentiate direct offspring and closely-related kin from more distantly-related conspecifics. Consequently, they cannibalise more divergent strains but not relatives. Our previous work has begun to elucidate the associated molecular mechanisms involved which include a small peptide SELF-1 which is essential for generating the *P. pacificus* kin signal. Moreover, we have recently shown that the presence and composition of the external surface lipids which coat *P. pacificus* are also necessary for this signalling processes. However, the mechanism by which such signals are detected by the organism remains unknown. Therefore, in an effort to identify the putative kin-recognition receptor, we have utilised recombinant inbred lines (RILs) generated between divergent strains which predate on one another and a quantitative trait loci (QTL) analysis which identified a single peak on chromosome II associated with this trait. Subsequently, we were able to narrow down the region of interest to less than 40 predicted protein coding genes. This interval also contains *self-1*, indicating close genetic linkage between kin-recognition signals and receptors. Five genes in this region are predicted to contain protein domains consistent with potential receptor function including several GPCRs. As such, we generated CRISPR/Cas9 mutants in all these candidate genes. Strikingly, mutations in one candidate located adjacent to *self-1* induced a kin-recognition defect consistent with abnormal kin-detection abilities but not kin-signalling revealing its role as a kin-recognition receptor. This receptor is expressed in a single pair of pharyngeal neurons which we have putatively identified as the *P. pacificus* I1 neurons. Further investigations will reveal the role and mechanisms of this receptor in mediating kin-recognition as well as determining the neural pathways that modulate the predatory behaviours in response to kin contact. Thus, we present first evidence of a kin-recognition receptor in nematodes.

131 The role of cAMP-Dependent Protein Kinase A Signaling in *C. elegans* Nociception and Nociceptive plasticity

Parvathi Sushama Gopinath, Aurore Jordan, Michael Stumpe, Joern Dengjel, Deiter Kressler, Dominique Glauser
University of Fribourg

Understanding the molecular and cellular mechanisms controlling nociception and its plasticity can reveal new therapeutic targets to treat human pain, including very prevalent and detrimental chronic pain conditions. cAMP-dependent Protein Kinase A (PKA) is a ubiquitously expressed protein with many biological functions, including, e.g, in neuropathic and bone cancer pain. However, the downstream pathways and phospho-substrate proteins through which PKA regulates nociceptive responses and plasticity are poorly understood. In *C. elegans*, the *kin-1* and *kin-2* genes encodes the catalytic and the regulatory subunit of PKA, respectively. Since the homozygous *kin-1* loss-of-function mutant is lethal, our lab employed a *kin-2* mutant in which KIN-1 is constitutively active. In noxious heat avoidance assays, *kin-2* mutants showed a striking phenotype. While naive wild type worms (never exposed to noxious heat) display robust heat-evoked reversals and reduce their response upon repeated stimuli, constitutively active PKA in *kin-2* mutants caused an opposite phenotype: reduced responsiveness in naïve worms and enhanced responsiveness after repeated stimulations. Therefore, PKA can either up-regulate or down-regulate thermo-nociceptive responses according to the context. To identify the downstream proteins, pathways and neurons controlled by PKA, we engaged two phosphoproteomics approaches. First, we identified potential targets of KIN-1 *in vivo*, as over-phosphorylated proteins in a *kin-2* mutant background as compared to wild-type (N2), via stable isotope labelling of the nematodes followed by phosphoproteomics. Second, we performed *in vitro* kinase assays where a *C. elegans* protein extract library was treated with purified KIN-1 to identify the phosphorylated substrates. Such *in vitro* kinase assays with three different KIN-1 isoforms, predominantly expressed in the nervous system, showed a conserved substrate consensus and identified around 600 different phosphosites in 500 different proteins. These candidate substrates were prioritized based on neuronal expression. Out of 171 mutants screened for thermo-nociceptive defect, we identified 32 mutants with affected responsiveness. We hypothesize that the regulation of these targets by PKA contributes to the peculiar phenotype of *kin-2* mutants. Ongoing epistasis analysis, rescue experiments and other neurogenetic approaches will help us further dissect the role of PKA and these downstream proteins in nociception and plasticity.

132 Eukaryotic Elongation Factor 2 Kinase EFK-1/eEF2K promotes starvation resistance by preventing oxidative damage in *C. elegans*

Junran Yan[1,2,3,4], Forum Bhanshali[1,4,5], Chiaki Shuzenji[1,2,4,5], Tsultrim T. Mendenhall[6], Shane K. B. Taylor[1,2,4,5], Glafira Ermakova[1,2,4,5], Jessica H. Hartman[6], Stefan Taubert[1,2,3,4,51]Centre for Molecular Medicine and Therapeutics, University of British Columbia, [2]Edwin S.H. Leong Centre for Healthy Aging, University of British Columbia, [3]Graduate Program in Cell & Developmental Biology, University of British Columbia, [4]British Columbia Children's Hospital Research Institute, University of British Columbia, [5]Department of Medical Genetics, University of British Columbia, [6]Department of Biochemistry and Molecular Biology, Medical University of South Carolina

Cells and organisms frequently experience starvation. In response, they mount an evolutionarily conserved stress response to adapt and survive. A vital component in the mammalian starvation response is eukaryotic elongation factor 2 (eEF2) kinase (eEF2K), which suppresses translation in starvation by phosphorylating and inactivating the translation elongation driver eEF2. In *C. elegans*, the eEF2K ortholog *efk-1* phosphorylates EEF-2/eEF2 on a conserved residue and is essential for L1 starvation survival, but how it promotes survival remains unclear. To study how *efk-1* promotes starvation resistance, we used the *efk-1(ok3609)* mutant which we confirmed was functionally null and had diminished *efk-1* mRNA expression and abolished EEF-2 T56 phosphorylation. Surprisingly, unlike in mammalian cells, EEF-2 T56 phosphorylation is constitutive in both fed and starved wild-type *C. elegans* and does not increase in starvation. Moreover, *C. elegans* strains engineered to lack EFK-1 kinase activity or EEF-2 T56 phosphorylation are indistinguishable from wild type with regards to their ability to survive and recover from starvation, suggesting an alternative, kinase-independent mechanism of *efk-1*. To map this noncanonical pathway, we identified two transcription factors, bZIP transcription factor family 2 (ZIP-2) and *C. elegans* p53-like protein 1 (CEP-1), which are also required for starvation resistance and function downstream in the *efk-1* pathway. Transcriptomic profiling of *efk-1*, *zip-2*, and *cep-1* mutants revealed that the three factors are jointly required for increased expression of DNA repair pathway genes during starvation. Specifically, *efk-1* is required to upregulate nucleotide excision repair (NER) and base excision repair (BER) to increase resistance to oxidative DNA damage, which is linked to starvation-induced oxidative stress. Additionally, *efk-1* prevents reactive oxygen species (ROS) accumulation in the cell, maintains mitochondrial physiology and morphology, and represses oxygen consumption, all of which may ameliorate oxidative stress during starvation. As these cytoprotective effects of *efk-1* are independent from translation elongation regulation and phosphorylation status of EEF-2, our studies reveal a noncanonical mechanism of *efk-1*-mediated starvation resistance.

133 A Nose for Trouble: Oxidant Detection in Olfactory Neurons Mediate Stress Responses in *C. elegans*

Victoria R Yarmey[1,2], Adriana San Miguel[21]Biomedical Engineering, NC State University, [2]Chemical and Biomolecular Engineering, NC State University

Reactive oxygen species (ROS) are small molecule radical species critical for cellular function and neuronal signaling. However, ROS accumulation due to oxidant exposure can cause mitochondrial dysfunction and cellular structure damage, which in turn can lead to cancer, inflammatory diseases, and neurodegeneration. Current research in *C. elegans* indicates that sensory neurons have roles in both the early detection of extracellular oxidants, such as H2O2, as well as in initiating subsequent behavioral responses and stress-resistance gene expression. Characterizing these responses, identifying their underlying mechanisms, and evaluating how they impact organismal health outcomes may thus identify novel targets for the prevention and treatment of oxidative diseases. We identified individual neurons responsible for detecting molecularly complex oxidants (juglone, paraquat, rotenone) then investigated the method and role neuronal oxidant detection serves in the host stress response. Using a combination of avoidance assays and olfactory calcium imaging, we establish that specific subsets of chemosensory neurons are responsible for detecting oxidants in a chemical- and concentration- specific manner. Additionally, through antioxidant expression and ROS quantification we determined that, for acute oxidant exposures, this detection serves to initiate behavioral rather than transcriptional responses. However, results of extended exposures suggest some expression differences exist between wildtype and detection-deficient mutants. Avoidance assays probing into the potential signaling pathways responsible for this phenomenon indicate that detection is due to a combination of both redox-dependent and standard chemosensory mechanisms. Based on this assumption, we computationally screened for highly expressed chemoreceptors, signaling molecules, and redox-sensitive proteins within oxidant-sensitive neurons according to their responsiveness by combining neuronal expression values from RNAseq databases (CeNGEN, WormSeq) with neuronal activity metrics generated from olfactory imaging. Together, our preliminary results suggest the existence of a complex strategy, involving the integration of multiple sensory cues, that determines whether or not an oxidant attack warrants escaping versus staying and/or mounting a transcriptional host defense.

134 LIN-39 functions as a neuron-specific developmental determinant of longevity in *Caenorhabditis elegans* with reduced insulin/IGF-like signaling

Alan Kavsek[1], Jerome Salignon[1], Lluis Millan-Arino[1], Ilke Sen[1], Christian G Riedel[1,2,31]Dept of Medicine Huddinge, Karolinska Institutet, [2]Dept of Molecular Biosciences, Stockholm University, [3]Faculty of Life Sciences: Food, Nutrition, and Health, University of Bayreuth

The nuclear chromatin landscape changes with age, a phenomenon that has been observed across many species including humans. Importantly, multiple studies have shown that perturbation of this landscape can be sufficient to change the rate of aging, indicating at least a partial role in controlling this process. This raises the question whether differences in the chromatin landscape are also a distinguishing feature of animals that age at unusual rates. Here we addressed this gap, by focusing on insulin/IGF-like signaling (IIS) as a well-characterized signaling pathway whose impairment drastically slows aging and extends the organism's lifespan. Specifically, we conducted ATAC- and mRNA-seq in *Caenorhabditis elegans* with reduced IIS, i.e. *daf-2* mutants. Interestingly, we found that while enhancer regions become closed and transcriptionally repressed with age they become opened and transcriptionally active under reduced IIS, indicating their potential relevance for lifespan regulation. By screening through proteins binding these regions, we identified LIN-39 as a new aging-preventive transcription factor (TF) required for the longevity of *daf-2* mutants. LIN-39 performs this role specifically during development and specifically in neurons. Further investigation showed that LIN-39 was acting around the L3 stage and in the hermaphrodite-specific VC class of cholinergic motor neurons. Finally, aging-prevention by LIN-39 was dependent on DAF-16/FOXO, an established pro-longevity TF residing downstream of DAF-2. This TF may synergize with LIN-39 in VC neurons and/or act downstream of their emitted signal. Ultimately, we propose a model whereby longevity of *daf-2* mutant hermaphrodites requires a longevity-promoting signal emitted by VC neurons – a signal that relies on correct VC neuron maturation around the L3 stage which is assured by LIN-39 and resulting chromatin and gene expression changes. Remarkably, this renders LIN-39 a rare example of a developmental determinant of longevity.

135 Tissue-specific roles for the miRNA Argonaute ALG-1 in healthy aging

Corrina Elder[1], Ian Nicastro[2], San Luc[2], Erin Schiksnis[2], Amy Pasquinelli[21]Biology, UC San Diego, [2]UC San Diego

Aging is a complex process influenced by a variety of genetic and environmental factors that are not yet fully understood. In *Caenorhabditis elegans*, the process of aging can be characterized by progressive declines in key physiological functions, including reproductive capacity, motility, and stress resistance. Previous research indicated that the miRNA Argonaute effector protein ALG-1 promotes longevity, as losing ALG-1 protein significantly reduces lifespan. Through transcriptional profiling of larval and adult *alg-1* genetic mutants, we've identified unique sets of misregulated protein-coding genes with very few genes shared between young and old worms. Interestingly, there is an enrichment of up-regulated neuronal-specific and intestinal-specific genes in the *alg-1* adult mutant. As previous work has shown that individual tissues play pivotal roles in regulating longevity, we investigated the tissue-specific roles of ALG-1 in aging by assessing its impact on germline structure, motility, pharyngeal pumping, and intestinal integrity. We used tissue-specific RNAi to deplete *alg-1* mRNA at the onset of adulthood in the intestine, neurons, body wall muscles, epidermis, distal tip cells, and germline and then quantified the tissue-specific contributions of ALG-1 to lifespan and healthspan. The loss of *alg-1* from the intestine alone led to a significant reduction in lifespan, intestinal integrity, and motility. To identify the molecular mechanisms behind these aging-related phenotypes, we sequenced the RNA of adult populations that lack ALG-1 in the whole body or in the intestine and analyzed differential gene expression compared to control populations. These analyses point to misregulation of a stress pathway that likely results in the rapid decline of adults lacking ALG-1 globally or just in the intestine. By elucidating the tissue-specific functions of ALG-1 and identifying key gene expression changes associated with its depletion, this work advances our understanding of how RNA-mediated regulation contributes to health and aging.

136 Adenylosuccinate Synthetase Is Required for Neuromuscular Health – Insights into Its Biological Functions and Implications in ADSSL1-Myopathy

Rishika R Patil, Melinda Jin, Latisha Franklin, Belle Barriga, Wendy Hanna-Rose Biochemistry and Molecular Biology, The Pennsylvania State University

Perturbations to purine metabolism are associated with congenital neurological and muscular disorders that are severely under-reported and often go undiagnosed or misdiagnosed due to a lack of awareness surrounding them. The molecular mechanisms driving the phenotypes due to these perturbations are poorly understood. Thus, these disorders pose significant challenges to medical diagnosis and treatment.

ADSSL1-Myopathy is a recently recognized ultra-rare muscular disorder caused by a mutation in the gene, ADSSL1. ADSSL1 encodes adenylosuccinate synthetase (ADSS), an enzyme in the Purine Nucleotide Cycle that plays a crucial role in energy metabolism in the muscle. Symptoms of the disorder include movement dysfunction, muscle weakness, lipid accumulation in the muscles, sensory disabilities, and the disruption of proper muscle structure. ADSSL1-Myopathy has not been investigated at a molecular level due to a lack of animal models for its study, hindering the progress towards effective therapeutic strategies to treat it. Therefore, we are investigating the biological functions of ADSS using a *C. elegans* deletion mutant of *adss-1*.

We have established that interference in the function of *adss-1* results in movement defects characterized by slower crawling speed, reduced thrashing rate, a shorter wavelength, and uncoordinated movement. To further investigate the phenotypes of slower and uncoordinated movement, we have probed the neuromuscular junction through paralysis assays using aldicarb and levamisole. Our findings indicate that *adss-1* mutants exhibit hypersensitivity to aldicarb and a dose-dependent resistance to levamisole. We have also observed defects in the organization of muscle fibers and aggregations of myosin. Based on the wavelength phenotype, we hypothesized that interference in ADSS function also results in defects of mechanosensation or proprioception. Through touch assays, we have shown that *adss-1* mutants exhibit a reduced ability to respond to external stimuli compared to the control. Additionally, *adss-1* mutants exhibit a developmental delay and defects in size. Overall, our work has shown that *C. elegans* is capable of recapitulating some of the phenotypes seen in human patients, supporting the use of it as a model for studying ADSSL1-Myopathy. This has provided a foundation for our current work to explore therapeutic strategies that involve supplementation with purines, blocking purine degradation, and supplying the absent enzyme product.

137 A novel cell non-autonomous mitochondrial stress response pathway mediated by gas sensing machinery

Rebecca Cornell, Wei Cao, Ava Handley, Roger Pocock Anatomy and Developmental Biology, Monash University

Maintaining mitochondrial function throughout the body is crucial for organismal health and survival. Mitochondrial stress responses are essential molecular mechanisms that maintain metabolic homeostasis. Local stress responses can be communicated to distal tissues to enable systemic reactions to challenges, thereby increasing the chance of survival. The nervous system is critical for coordinating stress responses across multiple tissues, yet the mechanisms by which this is achieved are not completely understood. In this study, we aimed to better understand how the nervous system controls distal mitochondrial function and health.

We previously showed that the ETS-5 transcription factor functions cell non-autonomously through the BAG sensory neurons to control intestinal fat storage. Increased intestinal fat levels in the absence of ETS-5 induces a sleep-like state known as quiescence. As sleep and metabolic alterations are hallmarks of systemic mitochondrial stress, the combination of increased fat storage and quiescence in an *ets-5* mutant implicates ETS-5 as a potential stress mediator. Our ensuing analysis revealed that ETS-5 indeed controls a highly specific mitochondrial stress response in the intestine. Specifically, loss of ETS-5 induces intestinal expression of the mitochondrial chaperone protein, HSP-6 (mortalin), but decreases intestinal HSP-60 expression, where typically the expression pattern of these chaperones would be mirrored. This non-canonical response has striking impacts on organismal and mitochondrial health, including increased resistance to mitochondrial stress and increased mitochondrial membrane potential. We have subsequently mapped a neuropeptide network utilised by the BAG neurons to influence systemic mitochondria, down to an intestinal receptor. Remarkably, this pathway is reliant on BAG neuron carbon dioxide sensing machinery. As animals utilise carbon dioxide sensing to detect viable food sources, this novel pathway likely represents an adaptive metabolic response to food scarcity.

138 RNA editing in response to mitochondrial dysfunction drives accelerated aging

Sean P. Curran[1], Carmen Ramos[2], Dilawar Mir[11]Gerontology, University of Southern California, [2]Molecular and Computational Biology, University of Southern California

The underlying basis of the variance in aging rates across individuals, tissues, and cells is often dismissed as a consequence of stochasticity. Muscle tissue is one of the most striking examples of age-related stochasticity where in a single animal, body wall muscle will contain visibly sarcopenic as well as healthy cells. Obviously, the nature of this stochasticity is not fully understood but what if the some of the variability in aging was tied to a genetic program responding to mitochondrial stress that drives molecular diversity? Adenosine deaminases (ADARs) are enzymes that catalyze the post-transcriptional deamination of adenosine (A) to inosine (I) on dsRNA substrates. This deamination can result in altered gene expression (editing of 5′ or 3′ UTR elements) or even altered codon usage in protein coding genes. Although several RNAs edited by ADARs have been previously reported, the biological significance of RNA editing and the impact of perturbing this conserved biological process are not fully understood. We have discovered that *adr-2*, the only catalytically active ADAR in *C. elegans*, mediates responses to mitochondrial stress across hierarchical levels, including molecular diversity of RNAs, mitochondrial homeostasis, muscle health, and organismal lifespan. Importantly, we find that *adr-2* is not required for the response to all environmental stresses (e.g., thermal, osmotic), which suggests that RNA editing is activated in a specific manner when mitochondria are impaired. Elucidating the role(s) of RNA editing by ADARs in the cellular responses to mitochondrial stress will aid in the development of future interventions to improve human health across the lifespan.

139 From Reproduction to Aging: Investigating the Physiological Roles of NAD+ Metabolism and Signaling

Abdulkareem A Alshaheeb[1], Jaedon Sadler[2], Ian A Argento[2], Shaina V Johnson[2], Cole Caron[2], Melanie McReynolds-[21]Biochemistry and Molecular Biology, The Pennsylvania State University, [2]The Pennsylvania State University

Promoting healthy aging has been a major focus of research for many years. Nicotinamide adenine dinucleotide (NAD+), in its oxidized form, plays a central role in cellular metabolism across all living organisms. It serves as a coenzyme in key redox reactions, including glycolysis, the TCA cycle, and oxidative phosphorylation, while also acting as a vital co-substrate for signaling enzymes such as PARPs, NADases (CD38 and CD157), and NAD+-dependent acetylases (Sirtuins). Together, these functions enable NAD+ to regulate essential cellular processes throughout the lifespan, making it a crucial factor in aging and overall metabolic health.

The age-related decline in NAD+ levels, observed across diverse model organisms, is a hallmark of metabolic aging and is linked to various metabolic disorders. Traditionally, this decline was attributed to either reduced synthesis, increased degradation, or a combination of both. However, recent studies revealed that the rate of new NAD+ synthesis remains unchanged in aged mice and that the decline is not due to limited precursor availability. Moreover, aged mouse tissues with lower NAD+ concentrations exhibit faster fractional turnover, maintaining total NAD+ flux. These findings suggest that increased NAD+ consumption, rather than impaired synthesis, is the primary driver of its age-related decline. Taking this together, the goal of this study was to gain deeper insights into the physiological functions of NAD+-consuming and signaling enzymes.

To investigate the role of NAD+-consuming and signaling enzymes in age-related NAD+ decline, we used a combination of genetic and molecular biology approaches alongside high-performance liquid chromatography-mass spectrometry (HPLC-MS) for precise metabolite quantification using *C. elegans.* Surprisingly, we identified novel reproductive and aging phenotypes associated with the loss of NAD+-consuming and signaling enzymes. Loss-of-function mutants exhibited significant reductions in both progeny numbers and lifespan. HPLC-MS analysis revealed dynamic shifts in metabolite levels across developmental stages, which were further supported by quantitative real-time PCR analysis of NAD+ synthesis and consuming genes. Furthermore, we conducted stable-isotope tracing with NAD+ precursors to examine how NAD+ flux varies with age. Together, these findings establish a previously unrecognized link between the hyperactivation of NAD+ degradation pathways and the onset of reproductive aging. This research underscores the critical role of NAD+ metabolism and signaling in sustaining reproductive health and lifespan, providing new insights into the intersection of metabolism and aging.

140 Raptor-RagA-Interacting residues control hypoxic sensitivity, development, and lifespan

Chun-Ling Sun[1], Cong Xu[1], Omar Itani[1], Elyse Christensen[2], Harshitha Vijay[1], Brock Grill[2,3,4], C. Michael Crowder[1,51]Anesthesiology and Pain Medicine, University of Washington, [2]Center for Integrative Brain Research, Seattle Children's Research Institute, [3]Department of Pharmacology, University of Washington, [4]Department of Pediatrics, University of Washington, [5]University of Washington

mTORC1 is a master regulator of anabolic metabolism, responding to nutrients and growth factors to promote cell growth. Inhibitors of mTORC1 are used as immunosuppressants and cancer chemotherapeutics and prolong lifespan of animals ranging from *C. elegans* to rodents. A severe limitation in the study of mTORC1 is the lack of viable loss-of-function alleles of metazoan mTORC1 subunits. In a forward mutagenesis screen for *C. elegans* mutants resistant to hypoxia-induced death, we isolated *gc67,* a viable missense mutation in *daf-15*, which encodes *C. elegans* raptor, a core subunit of mTORC1. Crispr/Cas9 engineering of *daf-15* (*daf-15*(*gc67* Crispr)) as well as *daf-15*(+) transgenic rescue confirmed that *daf-15*(*gc67*) produced hypoxia resistance. *daf-15*(*gc67*) phenotypes are temperature-sensitive: normal development, lifespan and hypoxic sensitivity when cultured at 20°, hypoxia resistant and extended lifespan at 22°, and a fully penetrant larval arrest at 25°. Temperature shift experiments show that *daf-15*(*gc67*) produces hypoxia resistance after development just prior to hypoxic exposure to protect from cellular injury whereas surprisingly lifespan extension is controlled during larval development. To define the pathway whereby raptor controls development and its other phenotypes, we screened 300,000 mutagenized *daf-15*(*gc67* Crispr) haploid genomes for mutants that develop to fertile adults at 25°. Whole genome sequencing and SNP-mapping identified twelve suppressors that contained second site *daf-15* intragenic mutations. All of the suppressors retained the original *gc67* mutation; Crispr/Cas9 engineering confirmed that these intragenic mutations were responsible for the suppression. The suppressors mutated four DAF-15 residues, one of which lay in the same helix as that altered by *gc67*, nine amino acids C-terminal. The other three residues were relatively distant from the *gc67* mutated residue. Alpha-fold modeling placed the remote suppressors on the same DAF-15 surface, shown by CryoEM of human mTORC1 to interact with RagA, a GTPase that recruits mTORC1 to the lysosome. We found that *raga-1*(lf) mutants were likewise hypoxia resistant and blocked suppression of hypoxia resistance and developmental arrest phenotypes. We conclude that *gc67* likely alters interactions between raptor and RagA that are critical for hypoxic sensitivity, development, and normal lifespan.

141 Molecular mechanisms of *Bacillus subtilis*-induced protection against □-synuclein aggregation in *Caenorhabditis elegans*

Deep Prakash[1], Samanta Paz Recio[2], Effy Guan[2], Fengqianrui Chen[2], Jamie Cousins[2], Liesa Salzer[3], Martin A. Schepers[4], Stefan Busscher[4], Kevin S. Chen[5], Kazuko Fujisawa[5], Michael Witting[3], Lorraine V. Kalia[5], Nicola Stanley Wall[6], Ellen Nollen[4], Maria Eugenia Goya[4], Maria Doitsidou[11]Centre for Discovery Brain Sciences, University of Edinburgh, [2]University of Edinburgh, [3]Research Unit Analytical BioGeo Chemistry, Helmholtz Zentrum München, [4]ERIBA, UMCG, University of Groningen, [5]Krembil Brain Institute, Toronto Western Hospital, University Health Network, [6]University of Dundee

Parkinson's disease (PD) is the second most common neurodegenerative condition. Accumulation of -synuclein (-syn) protein is a hallmark of PD and central to its pathogenesis. Increasing evidence suggests that gut bacteria influence PD progression and severity. However, the mechanisms through which the microbiota affects PD pathology remain unclear.

To investigate the effects of gut bacteria on PD pathology, we used a well-characterized *C. elegans* model that expresses human -syn fused to YFP in muscle cells (van Ham et al, 2008). On a standard *E. coli* OP50 diet, α-syn forms inclusions, which are visible via fluorescence microscopy. Previously, we showed that *Bacillus subtilis* PXN21, isolated from a commercially available probiotic product, inhibits, delays, and reverses α-syn aggregation and associated toxicity in the *C. elegans* model (Goya et al, 2020). The probiotic diet also mitigates α-syn-associated neurodegeneration and behavioral phenotypes.

To identify bacterial genes and metabolites that mediate the protective effect, we screened a genome reduction library of *B. subtilis 168* (Tanaka et al, 2013), covering 76% of the genome. In parallel we performed a genome-wide screen using a *B. subtilis* single-gene deletion library (Koo et al, 2017). Our results reveal that multiple bacterial pathways modulate α-syn aggregation, with genes involved in the purine biosynthesis, TCA cycle and energy metabolism being necessary for the protective effect.

In a complementary approach, we used comparative metabolomics to identify significant metabolite differences between protective and non-protective bacterial strains. We are investigating the potential protective role of candidate metabolites through supplementation experiments. Overall, our study highlights key bacterial pathways and metabolites that influence α-syn aggregation, with potential implications for disease-modifying interventions in PD.

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142 Endocytic recycling of DAF-2B in the nervous system

Bryan A Martinez, Jonathan I Hauser, Anne M Stene, Matthew S Gill Genetics, Cell Biology and Development, University of Minnesota

The *C. elegans* insulin / insulin-like growth factor signaling pathway is regulated by insulin-like peptides (ILPs) acting through DAF-2, the worm insulin/IGF receptor ortholog. We have previously found that DAF-2 activity is also affected by DAF-2B, a truncated, non-signaling isoform of DAF-2 that influences dauer formation and lifespan via sequestration of ILPs. We performed a forward genetic screen to identify regulators of DAF-2B protein expression, using a strain in which we tagged the endogenous DAF-2B locus with mScarlet, and determined that mutations in *unc-31* increased DAF-2B in the nervous system of adult animals. Mutants of *unc-31* have reduced secretion of neuropeptides, including ILPs, due to defects in dense core vesicle docking. However, DAF-2B secretion was increased in *unc-31* mutants, measured by accumulation in coelomocytes, indicating that elevated neuronal DAF-2B was not a consequence of defective exocytosis. On the other hand, neuronal DAF-2B levels were elevated by loss of the small GTPases *rab-5* and *rab-11.1,* suggesting that DAF-2B undergoes recycling endocytosis in the nervous system. Neuronal DAF-2B levels were also influenced by the availability of insulin-like peptides, since deletion of the antagonist *ins-18* and overexpression of the agonist *daf-28* decreased neuronal DAF-2B, while deletion of insulin-like peptide agonists increased DAF-2B. Our working model is that DAF-2B undergoes recycling endocytosis in the nervous system to clear insulin-like peptides.

143 Brain-Gut Tyramine signaling promotes bacterial immunity through HLH-30/TFEB-Mediated mitophagy in *C. elegans*

Yu-Chun Wu[1,2], Yu-Cheng Chang[1,2], Chun-Liang Pan[1,21]Institute of molecule medicine, College of Medicine, National Taiwan University, [2]Center for Precision Medicine, College of Medicine, National Taiwan University

In metazoans, intercellular regulation of mitochondrial morphology and function is important for physiological homeostasis at the organismal level. We previously found that perturbing neuronal mitochondrial dynamics in *C. elegans*, by using somatic CRISPR to delete the Mitofusin gene *fzo-1*, triggers systemic mitochondrial fragmentation in most somatic tissues, along with the induction of mitochondrial stress response. Here we show that neuronal *fzo-1* knockout (KO) triggers non-autonomous mitochondrial fragmentation in part through a brain-gut tyraminergic signaling circuit. Neuronal tyramine targets the TYRA-3 receptor in the intestine, which then activates EGL-30/Gq. Downstream of tyraminergic signaling, the master autophagy transcription factor HLH-30/TFEB translocates into the intestinal nuclei to induce mitochondrial fragmentation. In addition to activating the mitochondrial fission protein DRP-1, neuronal *fzo-1* KO also activates autophagy and mitophagy, via *tyra-3* and *hlh-30*. We found that the autophagy protein ATG-9 and mitophagy protein PDR-1/Parkin are recruited to the intestinal mitochondria upon *fzo-1* KO in the neurons. Neuronal *fzo-1* KO upregulates the transcription of the mitophagy gene *dct-1*/BNIP3, and this transcriptional activation required *hlh-30* through a conserved promoter sequence. Importantly, autophagy plays a key role in the enhanced resistance against the pathogen *Pseudomonas aeruginosa* conferred by non-autonomous mitochondrial fragmentation under neuronal *fzo-1* KO. Our study reveals molecular mechanisms that link neuronal mitochondrial stress to global mitochondrial dynamics and pathogen immunity. (Supported by National Science and Technology Council NSTC-113-2320-B-002-024-MY3 and National Health Research Institutes NHRI-EX113-11134NI)

144 Ribonuclease DIS-3 promotes longevity by generating tRNA halves that reduce translation via binding ribosomal proteins

Seokjun G. Ha[1], Jisoo Park[1], Hanseul Lee[1], Donghun Lee[1], Gee-Yoon Lee[1], Seokjin Ham[1], Sujeong Kwon[1], Sangsoon Park[1], Sieun S. Kim[1], Jongsun Lee[1], Jooyeon Sohn[1], Seon Woo A. An[2], Heehwa G. Son[1], Wooseon Hwang[2], Ok Hyun Park[3], Eunseok Kang[1], Yoon Ki Kim[1], Gwangrog Lee[1], Seung-Jae V. Lee[11]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, [2]Department of Life Sciences, Pohang University of Science and Technology, [3]Department of Life Sciences, Korea University

Transfer RNA (tRNA) halves (tRHs) are small RNAs that are generated by cleavage of tRNAs. However, whether tRNA halves regulate aging and longevity remains unknown. Here, we performed an RNAi screen targeting all 71 ribonuclease genes in *Caenorhabditis elegans*, and identified DIS-3/DIS3, a highly conserved ribonuclease, which mediated the generation of tRHs. Because *dis-3* null mutation caused early larval arrest, we focused on the four available *dis-3* missense mutants. Among them, *dis-3(L75Q)* mutation that affects the endonuclease domain of DIS-3, decreased the levels of 5′-tRH-Gln. We then showed that *dis-3* is required for longevity conferred by dietary restriction (DR) in *C. elegans*. We found that DR increased the levels of DIS-3 in the cytoplasm, where tRNAs are cleaved. We also found that overexpression of 5′-tRH-Gln, a product of DIS-3, was sufficient to extend lifespan. Thus, tRHs generated by DIS-3 in the cytosol appear to mediate longevity conferred by DR. Next, we performed RNA pulldown assays using synthesized 5′-tRH-Gln followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis, and identified seven ribosomal protein subunits as major 5′-tRH-Gln-binding proteins. Among them, RNAi targeting ribosomal protein large subunit 12 (RPL-12) restored DR-mediated longevity in *dis-3(L75Q)* mutants. We found that genetic inhibition of *dis-3* suppressed low translation in DR-mimetic *eat-2(ad1116)* mutants. These results suggest that 5′-tRH-Gln generated by DIS-3 binds ribosomal proteins to reduce translation, leading to longevity. Next, by performing RNA sequencing analysis, we found that *dis-3*-dependently upregulated genes in *eat-2(ad1116)* mutants significantly overlapped with those upregulated by PMK-1/p38 mitogen-activated protein kinase (p38 MAPK) and SKN-1/nuclear factor erythroid-related factor (NRF). In addition, genetic inhibition of *skn-1* did not further decrease the lifespan of *dis-3(L75Q)* mutants under DR condition, while suppressing the long lifespan of *eat-2(ad1116); dis-3(L75Q); rpl-12(RNAi)* animals. Thus, DIS-3 contributed to longevity conferred by DR by reducing translation in a SKN-1/NRF-dependent manner. Lastly, we showed that the tRH-generating and senescence-preventing roles of DIS3 were conserved in cultured human cells. Overall, our study indicates that DIS-3/DIS3 is an evolutionarily conserved tRH-generating ribonuclease that prevents premature aging at organismal and cellular levels.

145 Extracellular vesicle (EV) profiling in *C. elegans*: new insights into cargo selection and cellular communication

Inna Nikonorova, Elizabeth A. desRanleau, Katherine A. Jacobs, Josh A. Saul, Jonathon A. Walsh, Juan Wang, Maureen M. Barr Rutgers University

Extracellular vesicles (EVs) are essential mediators of cellular communication within an organism and across different biological kingdoms. Studying EV biology is challenging due to the diverse origins of EVs. Here, we used indirect proximity labeling to identify EV cargo associated with the transient receptor potential channel polycystin-2 PKD-2, a conserved EV cargo whose homologs in humans are implicated in ciliopathies, such as polycystic kidney disease.

In *C. elegans*, polycystins PKD-2 and LOV-1 form a complex that functions in the cilia of male-specific sensory neurons to regulate mating behavior. The polycystin complex is shed from the ciliary tip into the environment for inter-animal communication. Polycystin EV shedding can be observed in real-time using endogenous, fluorescently tagged proteins. The signaling mechanism and cargo of the polycystin-carrying ciliary EVs remain unknown.

We discovered that *C. elegans* polycystins associate with specific cargo on ciliary EVs: the polycystin-associated channel-like PACL-1, dorsal and ventral C-type polycystin-associated membrane lectins (PAML-1 and PAML-2), and conserved tumor necrosis-associated factor (TRAF) signaling adaptors TRF-1 and TRF-2. We found that EV cargo loading relied on polycystin-1 LOV-1. Loss of LOV-1 resulted in environmentally-released PKD-2 EVs lacking PACL-1, PAML-1 and -2, and TRAF signaling adaptors. These findings demonstrate how a single genetic perturbation can profoundly alter EV composition and impact cellular communication. (BioRxiv doi:10.1101/2024.04.17.588758, accepted in Nature Communications).

TRAFs are associated with ciliopathy-like phenotypes in mice. Since TRAFs emerged as conserved effectors of the polycystin signaling pathway, we next leveraged our indirect proximity labeling approach to identify additional interactors. For that, we pulled down intracellular partners of TRF-1, TRF-2, PKD-2, and LOV-1 from whole-worm lysates. Among the top candidates for each bait, we found previously validated EV cargo (PAMLs and PACL-1) alongside novel candidates. We are in the process of active investigation of how the novel candidates contribute to polycystin function in cilia and in EV-mediated signaling.

146 Lost in Translation No More: Midbodies as Hubs of RNA Activity and Large Extracellular Vesicle Biogenesis

Ahna Renee Skop Genetics, Univ Wisconsin-Madison

Our lab identified and characterized the role of the midbody (MB)-associated RNA-binding protein ATX-2 in early *C. elegans* embryos. One striking phenotype observed following ATX-2 knockdown was a significant delay in abscission, leading us to propose a radical hypothesis: translation may play a critical role in the final stages of mitosis. To explore this, we investigated RNA localization and activity within mammalian MBs. Historically considered vestigial remnants of cytokinesis, MBs and midbody remnants (MBRs) have recently emerged as central players in cell communication, fate determination, and extracellular vesicle (EV) biology. We discovered that the MB matrix functions as a hub for ribonucleoprotein assembly, enriched with mRNAs encoding proteins involved in cell fate, oncogenesis, and pluripotency—collectively termed the «MB granule.» Translation occurs within both MBs and post-abscission MBRs in a spatiotemporally regulated manner, initiating as nascent daughter cells transition into G1 and continuing after extracellular release. RNA localization to the MB dark zone is regulated by MKLP1 and ARC, while ESCRT-III modulates translation levels. Next, we established that MBRs represent a novel class of EVs by demonstrating that all commonly used EV isolation methods recover MBRs. Furthermore, inhibiting cytokinesis or cell cycle progression reduces MBR production as well as EV generation across all size classes, suggesting that proliferative cells are major contributors to EV biogenesis. Based on these findings, we propose a model linking EV production to cell cycle dynamics, highlighting mitotic cells as key sources of these actively translating large EVs. Our discovery of MBRs as translating EVs provides new insights into cancer diagnostics and therapeutic engineering while redefining our understanding of post-mitotic cellular processes.

147 Defining the impact of superoxide dismutase on extracellular vesicle biogenesis and cargo sorting

Nahin Siara Siara Prova[1], Jessica Siara Tanis[21]Biological Sciences, University of Delaware, [2]University of Delaware

Extracellular vesicles (EVs), released by nearly all cell types, mediate intercellular transport of protein, RNA, metabolite, and lipid cargo that cannot readily cross the plasma membrane. Dictated by the site of origin, the cargoes packaged into EVs regulate normal physiological processes and propagation of pathological conditions. The cellular factors that modulate EV biogenesis and the enrichment of cargoes into distinct EV subpopulations remain unclear. EVs are released from cilia of *C. elegans* male sensory neurons, then are taken up by surrounding glia or released into the environment. The ion channel CLHM-1 is packaged into EVs shed from the periciliary membrane compartment of the ciliary base, while the TRP polycystin channel PKD-2 is found in EVs that bud from the cilium distal tip. To define factors that impact EV cargo loading and biogenesis, we image and quantitate the number of EVs released from animals that express tdTomato-tagged CLHM-1 and GFP-tagged PKD-2 at single copy. Accumulation of reactive oxygen species (ROS) is a byproduct of both physiological and pathophysiological processes in eukaryotic cells. If not neutralized, aging or pathogen-induced ROS production could potentially disrupt EV biogenesis and cargo sorting. Superoxide dismutase (SOD-1) is an antioxidant enzyme that acts as a ROS scavenger to help reduce oxidative stress. I found that loss of *sod-1* affected shedding of EVs into the environment, increasing shedding of both CLHM-1 EVs from the ciliary base and PKD-2 EVs from cilium distal tip. Loss of *sod-1* differentially impacted ciliary abundance of the EV cargoes, enriching PKD-2 localization in the cilium proper while diminishing CLHM-1 localization in the ciliary base. Further, loss of *sod-1* reduced uptake of CLHM-1 EVs by the surrounding glial cells, which has potential to impact neuron-glia communication. In conclusion, we have found that loss of *sod-1* impacts both EV biogenesis and the abundance of EV cargos in ciliary subcompartments. This work is enabling us to achieve an understanding of how an increase in ROS affects EV biogenesis and cargo sorting, which is important because oxidative stress is an underlying factor for numerous pathological conditions that are impacted by EV release.

148 From the filament to the tissue: the impact of myosin filament stoichiometry on embryonic morphogenesis.

Daniel Plura, Karen Chauca, Teresa Ferraro, Flora Llense, Francois RobinDEV2A, Sorbonne (IBPS)

The interplay between biochemistry and mechanical forces is fundamental to cellular and tissue morphogenesis, and the actomyosin cortex is a central player in driving shape changes. While it is well established that mechanical forces generated by non-muscle myosin II (NMII) are critical for these processes, a thorough understanding of how these forces are tuned and regulated to control cell or tissue mechanics remains elusive. In particular, the interplay between NMII diversity and the dynamic changes of mechanical properties that govern cell shape is poorly understood. To address this issue, we focused on how the two NMII isoforms of C. elegans, NMY-1 and NMY-2, individually contribute to embryonic morphogenesis and elongation. During this process, two key tissues –seam cells and dorso-ventral (DV) cells– cooperate to modulate cell mechanics in a tissue-dependent manner, driving elongation along the anterior-posterior axis. To determine whether NMY-1 and NMY-2 play distinct roles in elongation, we performed isoform-specific depletion using the nmy-1(sb115) knockout strain and the nmy-2(ts) strain. Loss of NMY-2 allowed embryos to elongate fully but led to a 40% increase in lethality during this stage, suggesting that the structural integrity of the actomyosin cytoskeleton is compromised. In contrast, NMY-1 depletion did not affect elongation initiation, and NMY-1-depleted embryos failed to complete elongation, arresting at 3-fold. These results highlight that NMY-1 and NMY-2 are not redundant but instead have complementary functions in ensuring successful elongation. To reveal the molecular bases of the specific roles of NMY-1 and NMY-2 during elongation, building on previous work in reconstituted systems, we developed tools to associate filament composition with their specific dynamic and motility. We used isoform-specific tagged NMII and live super-resolution SIM-TIRF microscopy to assess filament stoichiometry in vivo. Strikingly, we observed that NMY-1 and NMY-2 were able to assemble into mixed filaments, and that these filaments exhibited cell-specific stoichiometries, with NMY-1-enriched minifilaments in the seam cells and NMY-2–rich predominant in DV cells. In particular, we could show that homotypic NMY-2 filaments have significantly shorter cortical dwell times than mixed filaments, suggesting that isoform composition can regulate filament stability. Altogether, our findings underscore the first in vivo study of division of labor between NMII isoforms, shedding light on how mechanical forces can be tuned and balanced to drive elongation in C.elegans, and provides an in vivo model system to explore how changes in relative myosin isoform stoichiometries affect filament dynamics, motile properties, and overall cell mechanics.

149 GENTIS: A Nuclear Translocation Biosensor for Intracellular Ionic Stress in Live *C. elegans*

Zhijian Ji, Zhijian Ji Cardiovascular research institute UCSF

Maintenance of ionic homeostasis is fundamental to cellular function, yet tools for dynamically monitoring ionic stress responses *in vivo* remain limited. To address this, we developed GENTIS (Genetically Encoded Nuclear Translocation Ionic Sensor), a genetically encoded biosensor that enables real-time detection of intracellular ionic strength changes in living systems. GENTIS integrates a charge-sensitive helical dimer fused to GFP, regulated by competitive nuclear localization/export signals, to report ionic fluctuations via nucleocytoplasmic shuttling. We demonstrate that GENTIS responds to ionic stress triggered by hypertonic agents (sorbitol, glucose, NaCl), non-osmotic ionic challenges (NH4OAc), with nuclear translocation reflecting ionic strength increases independent of phase separation. In *C. elegans*, GENTIS reveals developmentally programmed nuclear accumulation during larval transitions, implicating endogenous ionic regulation in larva development. RNAi of importin-α (*ima-3*) and importin-β (*imb-1*) and genetic disruption of *xpo-2* abolished stress-induced and developmental specific translocation, confirming canonical transport dependency. By bridging ionic dynamics with subcellular trafficking, GENTIS provides a versatile platform to dissect stress adaptation mechanisms, developmental ionic signaling, and disease-associated homeostatic dysfunction.

150 SPD-5 forms a dormant MTOC halo around sperm DNA

Jeremy Magescas, Rachel K Y Ng, Jessica L Feldman Biology, Stanford University

During cell division, the centrosome acts as a microtubule organizing center (MTOC), nucleating and localizing microtubules through its pericentriolar material (PCM). In *C. elegans*, the PCM is composed of three essential proteins, PCMD-1, SPD-2/Cep-192, and SPD-5, the functional homologue of CDK5RAP2/Cnn. We previously found that although these proteins are all required during the first cell division in *C. elegans*, only SPD-5 is essential for MTOC function at the centrosome across cell types in the developing organism. Given this essential role of SPD-5 in MTOC function, we were surprised to find a novel localization of SPD-5 forming a “halo” surrounding the DNA in mature sperm as previous studies have shown that microtubules are removed from spermatocytes at the end of meiosis II: during anaphase, PCM and attached microtubules move into the residual body (RB), a cellular compartment that will be eliminated. Live imaging and expansion microscopy during halo formation revealed that soon after microtubules and attached PCM were stripped from the centrosome and removed to the RB, pools of uncleared SPD-5 progressively surrounded the sperm DNA, generating the halo. Although tubulin, and the microtubule regulators ASPM-1, EBP-2, KLP-7, and the g-TuRC component GIP-1 followed a similar pattern of halo localization, we have not observed evidence of dynamic microtubules. Thus, the halo localizes proteins essential for MTOC function at the centrosome, but does not appear to behave as an MTOC. We are confirming this hypothesis and assessing the structural nature of the halo using a combination of expansion microscopy and cryotomography. Sperm-specific SPD-5 depletion revealed that, as at the centrosome, SPD-5 is required for the recruitment of g-TuRC and tubulin and preliminary studies indicate that paternal SPD-5 is required for embryonic viability, suggesting a role for the halo in the zygote. Thus, the halo appears to represent a novel SPD-5-based structure that does not associate with dynamic microtubules, providing the opportunity to better understand how SPD-5 regulates microtubule assembly and dynamics and how these processes might be modulated during development.

151 RAB-35 regulates distinct steps of trogocytosis in the biting and bitten cell

Julie E Manikas[1], Yusuff Abdu[1], Liam Popovsky[2], Jeremy Nance[21]Developmental Genetics, NYU School of Medicine, [2]University of Wisconsin - Madison

A specialized form of cell-cell interaction called trogocytosis occurs when one cell "bites" off and internalizes pieces of another cell. During *C. elegans* embryogenesis, primordial germ cells (PGCs) form cellular extensions called lobes, which are "bitten" off and subsequently digested by adjacent endodermal cells. We previously showed that proteins involved in membrane scission, including dynamin and the sorting nexin LST-4/SNX9, function within the biting endodermal cells to cut off PGC lobes. Here, we performed a genetic screen to identify mutants with PGC lobes that fail to be removed. The screen identified multiple mutant alleles of the *rab-35* gene, which encodes a Rab GTPase involved in trafficking between endosomes and the plasma membrane. Using FRAP to determine if persistent lobes remained connected to the PGC cell body (a scission defect) or were disconnected (a digestion defect), we found that RAB-35 regulates both scission and digestion of PGC lobes. By degrading degron-tagged RAB-35 in specific cell types, we identified distinct cellular roles for RAB-35 in trogocytosis – endodermal cell depletion resulted in persistent lobes with a digestion phenotype, whereas PGC depletion caused scission as well as digestion phenotypes. These experiments suggest that the bitten cell plays an active role in regulating its own trogocytosis. We now aim to identify downstream RAB-35 effectors to elucidate how RAB-35 regulates scission and digestion during trogocytosis.

152 Single cell transcriptomics identifies MYRF-1/Myelin-regulatory factor as a novel transcriptional regulator of non-apoptotic cell death

Olga Yarychkivska, Simin Liu, Shai Shaham Developmental Genetics, The Rockefeller University

Migration and cell death are important for normal development and are deregulated in cancer, especially in the process of metastasis, which contributes to 90% of cancer deaths. How cell migration and death are intertwined in development and in disease is not well understood. To address this question, we study the *C. elegans* male linker cell: a migrating leader cell that undergoes LCD (Linker Cell-type Death), a novel non-apoptotic morphologically conserved cell death program, at the end of its migration. To identify new migration and LCD regulators, we performed 10x single-cell RNA sequencing on FACS-purified migrating and dying linker cells, focusing on 257 genes upregulated in the dying linker cell. These genes might promote cell migration termination and/or activation of LCD. As most of these genes are essential, we performed functional studies using linker cell-specific RNAi and scored animals for abnormal linker cell survival. We identified several novel classes of LCD regulators whose inactivation promotes inappropriate survival. These include chromatin factors, ESCRT III components, and a highly conserved gene, *myrf-1*, encoding a membrane-anchored transcription factor. Auxin-inducible degron depletion of MYRF-1 blocked LCD in ~70% of animals. Previous studies of *C. elegans* DD neurons revealed that PAN-1, a trans-membrane protein, recruits MYRF-1 to the cell membrane, inducing MYRF-1 trimerization, autocleavage, and translocation of the N-terminal cleavage product to the nucleus. Importantly, we showed that like *myrf-1*(RNAi), *pan-1*(RNAi) also promotes inappropriate linker cell survival, suggesting that PAN-1 may regulate MYRF-1 in the linker cell. Supporting this idea, we showed that GFP-tagged MYRF-1 translocates to the nucleus during LCD. Of note, 67% (172/257) of the genes upregulated in dying linker cells harbor MYRF-1 binding motifs. These include previously known LCD regulators, as well as new ones identified in our current work, suggesting that MYRF-1 likely transcriptionally regulates LCD. Previous work from our lab showed an important role for the HSF-1 transcription factor in LCD initiation, raising the possibility that HSF-1 and MYRF-1 might cooperate to induce cell death. By understanding how the migrating linker cell initiates its programmed death pathway, we hope to determine whether migration cessation and LCD are functionally connected, a finding that may, in the long run, help us understand the aggressiveness of metastatic cancers.

153 Notch activity is modulated by the Adhesion GPCR Latrophilin binding to the Notch ligand LAG-2

Willem Berend Post[1], Victoria E Groß[1], Daniel Matúš[2], Anette Kaiser[3], Fabian Ließmann[4], Iannis Charnay[1], Jens Meiler[5,6,7], Torsten Schöneberg[2,8], Simone Prömel[11]Institute of Cell Biology, Heinrich Heine University Düsseldorf, [2]Rudolf Schönheimer Institute of Biochemistry, Leipzig University, [3]Department of Anesthesiology and Intensive Care, University of Leipzig Medical Center (UKL), [4]Institute for Drug Development, Leipzig University, [5]Institute for Drug Development, [6]Center for Scalable Data Analytics and Artificial Intelligence ScaDS.AI, [7]Department of Chemistry, Center for Structural Biology, Vanderbilt University, [8]School of Medicine, University of Global Health Equity

The Notch pathway is essential to control cell proliferation and differentiation. Skewed Notch activity can lead to abnormal cell division and tumor formation, highlighting the need for proper modulation of this conserved pathway. In the *Caenorhabditis elegans* germline, the Notch receptor GLP-1 is activated by interacting with its somatically expressed ligand LAG-2. We found that the homolog for the Adhesion GPCR (aGPCR) Latrophilin (LAT-1) is able to modulate the activity of the Notch pathway. In the absence of the receptor, a decrease in germ cells as well as mitotic cells is observed. Using a Notch activation assay, we observed that this reduction is due to a lower GLP-1 activation, indicating that LAT-1 is involved in the initial stages of the Notch pathway.

Adhesion GPCRs (aGPCRs) contain characteristic long N termini are able to mediate a large variety of cell-cell and cell-matrix signals. This led us to hypothesize that the N terminus of LAT-1 might interact with either LAG-2 or GLP-1, modulating the pathway. Expressional analyses showed LAT-1 is expressed both on germ cells as well as in the Distal Tip Cell (DTC), making interactions with LAG-2 or GLP-1 feasible. Molecular modeling indicated that an interaction between LAT-1 and LAG-2 is likely, which we confirmed *in vitro* using BRET assays. We confirmed this interaction *in vivo* using BiFC (Bimolecular Fluorescence Complementation). Additionally, we ectopically expressed LAT-1 on the DTC and germ cells, indicating the interaction occurs on the DTC.

In summary, we have shown that the Latrophilin homolog LAT-1 modulates germ cell proliferation by cross-talking with the Notch pathway, interacting with the Notch ligand LAG2. This modulation balances the germ cell pool and might play a role for several other Notch functions in the nematode.

154 RME-1-associated Recycling Endosomes Participate in Vitellogenin Secretion in *Caenorhabditis elegans*

Chao Zhai[1], Meng-Qiu Dong[1,21]National Institute of Biological Sciences, Beijing, [2]Tsinghua Institute of Multidisciplinary Biomedical Research, Tsinghua University

It was demonstrated over four decades ago that in *C. elegans* the lipoprotein precursors of yolk proteins, vitellogenins (VITs), are expressed in the intestine, secreted into the pseudocoelom, and finally transported into oocytes, but the mechanism by which VITs are secreted from the intestine remains uncertain. Here, an RNAi screen of 79 known vesicular trafficking genes in VIT-2::GFP-expressing worms revealed that the conventional secretion pathway and recycling endosomes (REs) are both necessary for VIT secretion. Further RNAi assays with immuno-electron and confocal microscopy verified that VITs are synthesized in the intestinal rough ER, then transported to the Golgi apparatus. VIT-2::GFP signal could be detected within enlarged REs that form upon depletion of RME-1, which facilitate endocytic recycling. Moreover, knockdown of endocytosis-related genes decreased the number of VIT-2::GFP+ REs, as did inhibition of ER-to-Golgi or trans-Golgi-to-endosome vesicular trafficking, suggesting that REs are required for intestinal secretion of endocytosed yolk proteins and newly-synthesized VITs. Under standard culture conditions, we found that RME-1::RFP puncta consistently localized at the periphery of VIT-2::GFP+ vitellogenin-containing vesicles (VVs), suggesting that REs are closely associated with VVs. Additionally, we found that the upstream regulator of RME-1 in endocytic recycling, RAB-10, is required for RME-1 localization at the VV periphery and VV trafficking from the apical/luminal side to the basal/pseudocoelomic side of the intestine. This study identifies REs as an intermediate compartment for VIT secretion in the *C. elegans* intestine, thus providing a basis for exploring the secretion routes of lipid transfer proteins in mammals.

155 Non-canonical V-ATPase complexes regulate polarized membrane biogenesis

Liakot Khan, Edward Membreno, Gholamali Jafari, Caleb Sharif, Verena Gobel Massachusetts General Hospital and Harvard Medical School

V-ATPases are highly conserved, membrane-based proton pumps that acidify membrane-bound intracellular and extracellular compartments. They form multi-subunit complexes, typically composed of 13 subunits, arranged into a membrane-embedded VO and a cytoplasmic V1 domain. V-ATPases impinge on numerous physiological processes via their function in pH regulation, but they also have less well understood roles in the dynamics of endo- and plasma membranes. The prototypic yeast vacuolar H+-ATPase was identified by its role in the biogenesis of the yeast vacuole. Remarkably, the yeast VO domain promotes vacuolar membrane fusion (H+-independently), while the holoenzyme (VO&V1) regulates membrane fission (H+-dependently). This finding, made possible by subunit isoform distinct V-ATPases at the yeast Golgi and vacuole, has remained the only report of an antagonistic behavior of different components of this multi-subunit complex. Here, we report that multiple V-ATPase subunits reside at different endo- and plasma membrane domains in the *C. elegans* excretory canal and act antagonistically in intracellular apical membrane (lumen) biogenesis.

We carried out a systematic V-ATPase subunit/subunit isoform analysis in this single-cell tube. This analysis revealed that most VO domain subunits exclusively localize to, but only partially colocalize at, canalicular endo-membranes, whereas V1 domain subunits localize to (and colocalize at) the apical (lumenal) plasma membrane but are absent from endo-membranes. Of note, most subunits have only one isoform and would thus be expected to colocalize with all other domain subunits. Moreover, the loss of several canalicular VO subunits results in little or no lumenal membrane expansion, whereas the loss of several V1 subunits produces excess lumenal membrane. In agreement with co-localization studies in canals double-labeled with different subunit fluorophore fusions, a tripartite split-GFP assay, established to trace subunit interactions *in situ*, confirmed that several VO and V1 subunits failed to associate *in vivo*. These findings challenge the assumption of an obligate VO and VO&V1 assembly of this multi-subunit protein complex. Our results suggest that incomplete complexes form on different membrane domains and have antagonistic functions in polarized (apical) membrane biogenesis that balance the delivery of lumenal membrane required to extend the lumen inside the *C. elegans* excretory canal.

156 Kinetochore dynein is sufficient to biorient chromosomes and remodel the outer kinetochore

Bram Prevo[1], Dhanya K. Cheerambathur[1], William C. Earnshaw[1], Arshad Desai[21]University of Edinburgh, [2]University of California, San Diego

Faithful chromosome segregation requires the coordinated interplay between multiple microtubule motors and couplers at kinetochores in order to establish stable attachments and prevent aneuploidy. While the cytoplasmic dynein motor has been implicated in this process since its discovery at kinetochores in 1989, its precise role in ensuring accurate segregation across metazoans remains unresolved, hindered by functional redundancy and overlapping mechanisms.

To dissect dynein's contribution, we developed an innovative *in vivo* reconstruction approach in *C. elegans* embryos. Selectively isolating conserved microtubule-directed activities — of dynein, chromokinesin, and the kinetochore microtubule coupler Ndc80 — on mitotic chromosomes. Strikingly, dynein alone proved sufficient to mediate chromosome biorientation, a process that aligns sister chromatids toward opposite spindle poles. In contrast, dynein could not drive chromosome congression to the metaphase plate, a role primarily mediated by chromokinesin and the Ndc80 module. Furthermore, by simultaneously monitoring chromosome behavior and outer kinetochore component dynamics, we demonstrated that the kinetochore dynein dynamically remodels the outer kinetochore, removing components and itself in an attachment state-dependent manner. Thus, our findings suggest that dynein plays a critical role in establishing end-on microtubule attachments and resolving erroneous connections during biorientation and kinetochore maturation.

Beyond providing insights into the specific role of the kinetochore dynein module, our analysis using the *in vivo* reconstruction approach has shed light on the temporal coordination of the distinct microtubule-associated activities at mitotic chromosomes. This work establishes a mechanistic framework for understanding how conserved, yet modular systems have evolved to safeguard genome integrity across species.

157 A small N-terminal region of the histone kinase HASP-1 is essential for its role in chromosome segregation in meiosis

India Rosario, Olivia Gomes, Faith Crane, David J Wynne University of Portland

Precise segregation of chromosomes is a vital step of cell division because aneuploidy is common in cancer cells and can lead to inviable embryos. Haspin is a histone kinase that promotes correct chromosome segregation but is not as well understood as other mitotic kinases. Haspin is thought to function exclusively by promoting the recruitment of the Chromosomal Passenger Complex (CPC), which promotes correct chromosome segregation by multiple mechanisms. There is evidence that pools of CPC recruited by different mechanisms have different functions, but this observation is not yet well understood. Further, the presence of separate functional CPC pools has not been tested on meiotic chromosomes, which have dramatically different spatial organization than in mitosis. Investigating the role of Haspin in meiotic cell division has the potential to help understand the functions of CPC pools and determine if there are alterations in this circuit during meiosis. To better understand the function of Haspin, we are investigating the mechanisms by which it is activated and recruited to chromosomes. Haspin's localization to chromosomes is influenced by multiple interacting proteins and its kinase activity is regulated by multisite phosphorylation and an autoinhibitory sequence. Like in other organisms, the *C. elegans* Haspin homolog HASP-1 has its kinase domain at its C terminus, while the remainder of the protein is a long, disordered region without obvious domains. To test the mechanisms by which HASP-1 is recruited to chromosomes and activated during meiosis, we generated a series of deletions in the long N-terminal region of HASP-1. Deletion of residues 1-37 displayed a moderate decrease in embryonic viability, while deletion of residues 1-129 resulted in no viable embryos, phenotypes consistent with errors in chromosome separation of increasing severity. We identified putative phosphorylation sites in the deleted regions, and we generated point mutations to determine whether these sites are necessary to activate HASP-1. We are analyzing GFP labeled AIR-2, the kinase subunit of the CPC, to determine the effects of N-terminal HASP-1 mutations on recruitment of the CPC. By gaining insight into the regulation of both HASP-1 and the CPC, we can further understand how HASP-1 dependent and independent CPC recruitment mechanisms affect correct chromosome segregation in meiosis.

158 Dynamic Control of Argonautes by a Rapidly Evolving Immunological Switch

Chee Kiang Ewe[1], Guy Teichman[1], Shir Weiss[1], Maximilian Knott[1], Sarit Anava[1], Hila Gingold[1], Mario Bardan Sarmiento[2], Emily Troemel[2], Oded Rechavi[11]Tel Aviv University, [2]UCSD

Small RNAs, coupled with Argonuate proteins (AGOs), regulate diverse biological processes, including immunity against nucleic acid parasites. *C. elegans* possesses an expanded repertoire of at least 19 AGOs functioning in an intricate gene regulatory network. Despite their crucial roles, little is known about the regulation of AGOs, and whether their expression levels, tissue specificity, and functions change in response to genetic perturbations or environmental triggers. Here, we report that PALS-22, a member of an unusually expanded protein family in *C. elegans*, acts as a negative regulator of antiviral RNAi involving the RIG-I homolog. The loss of *pals-22* leads to enhanced silencing of transgenes and endogenous dsRNAs. We found that PALS-22 normally suppresses the expression of two AGOs, VSRA-1 and SAGO-2, which are activated by bZIP transcription factor ZIP-1. When *pals-22* is eliminated, *vsra-1* and *sago-2* are upregulated. These AGOs in turn play key roles in defense against foreign genetic elements and intracellular pathogens, respectively. Surprisingly, while in *pals-22* mutants immune genes functioning in the intracellular pathogen response (IPR) are upregulated, removing SAGO-2 or the RNA-dependent RNA polymerase RRF-3 in these mutants leads to the downregulation of these genes. This observation contrasts with the typical gene-silencing role of siRNAs. Finally, by analyzing *C. elegans* wild isolates and lab reference strains, we demonstrate that PALS-22 regulates the expression of several germline AGOs, affecting germline mortality and transgenerational epigenetic inheritance. In summary, PALS-22 is a key genetic node that balances the trade-off between immunity and germline health by modulating the functions of different AGOs, thereby shaping the outputs of the RNAi machinery and the dynamics of epigenetic inheritance.

159 Pleiotropic triggers underlying larval arrest in *mutator*-class mutants

Rhiannon Maddock, Alicia K. Rogers Department of Biology, University of Texas Arlington

For an organism to exist and persist, it must be able to robustly execute cellular and developmental programs, despite routine genetic and environmental stressors. All eukaryotes use the highly conserved RNA interference (RNAi) pathways to regulate their genomes in a precise, temporal, and cell-specific manner. Highly conserved membrane-less perinuclear germ granules are hubs for the cytoplasmic localization of RNAi factors and are a hallmark of germ cell identity in most, if not all, metazoans. In *C. elegans*, one critical process that occurs within a germ granule, called the *Mutator* focus, is *mutator* complex-dependent amplification of small interfering RNAs (siRNAs). Previously, we found that *mutator*-class mutants exhibit a low penetrance larval arrest phenotype that is exacerbated by L1 starvation. However, the underlying causes of the larval arrest in *mutator*-class mutants remained elusive and it was not known if the arrested larvae state was temporary (i.e. the larvae could eventually mature into adults). Interestingly, we found *mutator*-class mutants that arrest as larvae live shortened lifespans and never progress into adulthood. We sought to determine if the larval arrest phenotype was triggered by the animals' inability to consume food, metabolic defects, or due to the dysregulation of developmental pathways. Here we show that a subset of the arrested *mutator*-class larvae do not consume food and exhibit developmental defects, while another subset can consume food but are unable to progress into adulthood, potentially due to metabolic defects. The pleiotropic triggers of the larval arrest phenotype in *mutator*-class mutants indicates that RNAi plays a critical role in regulating several distinct developmental pathways whose molecular consequences converge to drive the arrest phenotype.

160 Maternal histone mRNA stabilization by piRNA biogenesis factors

Joana Pereirinha[1], Anke Busch[1], Ann-Sophie Seistrup[1], Nadezda Podvalnaya[1], Kamila Delaney[2], Florian Steiner[2], Julian König[1,3], Rene Ketting[11]Institute of Molecular Biology, Mainz, [2]Dept. of Molecular and Cellular Biology, University of Geneva, [3]Julius Maximilian University of Würzburg

Embryogenesis relies on maternally provided mRNAs and proteins, which sustain early development before zygotic genome activation. Transcripts from replication-dependent histones (RDH) are also thus provided to the embryo. This is striking, as RDH transcripts are normally unstable outside S-phase. How, then, can they be inherited maternally?

We previously identified PETISCO, a core component of *C. elegans* piRNAs biogenesis. Unexpectedly, it is critical for embryogenesis and leads to a Maternal-effect lethal (Mel) phenotype when absent, while maternal piRNAs are not required for embryogenesis. PETISCO interacts with one of two ligands: PID-1 and TOST-1. While PID-1 mediates PETISCO's piRNA biogenesis function, by stabilizing piRNA precursors, *tost-1* mutants do not affect piRNAs but display a Mel phenotype. Curiously, the PETISCO Mel phenotype closely resembles the phenotype triggered by RNAi against RDH genes.

We found that PETISCO specifically binds to all RDH mRNAs, predominantly at their conserved stem-loop region. This stem-loop is a key site for RDH transcript regulation: by binding to stem-loop-binding protein (CDL-1 in *C. elegans*) it stabilizes RDH mRNAs during S-phase and triggers their decay outside S-phase. Interestingly, CDL-1 does not co-immunoprecipitate with PETISCO, suggesting that PETISCO may replace CDL-1 on maternal RDH mRNAs. Loss of PETISCO function leads to RDH mRNA loss in adults and early embryos. Consistently, *tost-1* mutants exhibit premature gene activation during embryogenesis, a known consequence of insufficient RDH expression. Furthermore, deletion of one histone gene cluster enhances the Mel phenotype of a hypomorphic *tost-1* mutant, while impairing RDH mRNA decay can partially rescue it. Finally, a forward genetic screen revealed that histone protein stabilization can also rescue the *tost-1* Mel phenotype, strongly suggesting that histone-insufficiency is the main cause behind the PETISCO Mel phenotype.

Unlike their canonical cell cycle-dependent regulation, RDH mRNAs in the germline must be stabilized for successful transmission to the embryo. Our findings identify PETISCO as a key factor that mediates the thus-far unexplained maternal RDH mRNA stability. Evolutionary analyses suggest that PETISCO's histone mRNA stabilization function predates its role in piRNA biogenesis, suggesting that the piRNA pathway adopted an ancestral mRNA stabilization mechanism to stabilize another intrinsically unstable transcript: piRNA precursors.

161 Heterochromatin readers CEC-3 and CEC-6 regulate the duration of transgenerational epigenetic inheritance in response to heat stress.

Phoebe A. W. Bhagoutie, Chengyin Li, Victor Lao, Arneet L Saltzman Department of Cell and Systems Biology, University of Toronto

Transgenerational epigenetic inheritance (TEI) can be defined as heritable changes in gene expression that occur without modification to the genome sequence itself. Proper regulation of TEI can have important implications for an organism's ability transmit memories of past experiences to the next generation. In *C. elegans,* small RNA and chromatin pathways that regulate epigenetic memory can be essential for promoting germline health and fertility. Two previously characterized *C. elegans* Chromodomain containing (CEC) proteins, CEC-3 and CEC-6, safeguard the germline in a temperature-sensitive manner. To investigate the roles of *cec-3* and *cec-6* in regulating TEI, RNA interference (RNAi) inheritance assays using a germline *gfp* reporter were performed at three temperatures: 15°C, 21°C and 25°C. Mirroring the effect of temperature on germline immortality in *cec-3Δ;cec-6Δ* mutants, silencing inheritance was enhanced at elevated temperatures (25°C) compared to wildtype. Furthermore, GFP silencing is maintained in *cec-3Δ;cec-6Δ* mutants but not in wildtype worms when shifted from low to high temperatures (15°C to 25°C) in the F1 generation. These results suggest that heat stress can reset GFP expression in the generations post-RNAi exposure. Interestingly, the presence of cec-6 in the P0 generation and maternally in the F1 is sufficient to reset GFP expression indicating a role during the establishment phase of RNAi inheritance. In addition, *cec-3* and *cec-6* single mutants showed reduced or increased inheritance of silencing to the F1 generation, respectively, which suggests antagonistic roles. Maintenance of silencing inheritance in *cec-3Δ;cec-6Δ* mutants also depends on the germline nuclear Argonaute *hrde-1* and the Z-granule component *znfx-1,* but not the H3K9 methyltransferase *set-25.* Together these findings reveal the prospective roles of *cec-3* and *cec-6* in preserving the germline in response to heat stress by regulating the resetting of TEI in a nuclear RNAi-dependent manner.

162 The unusual case of miRNAs in transgenerational epigenetic inheritance of the predatory mouth-form

Shiela Pearl Quiobe, Ralf J Sommer Dept. for Integrative Evolutionary Biology, Max Planck Institute for Biology

Long-term environmental induction experiments of *Pristionchus pacificus* highlighted the role of ubiquitin ligase EBAX-1/ZSWIM8 and microRNA (miRNA) signalling in transgenerational epigenetic inheritance (TEI) of the predatory mouth-form. Specifically, we previously showed that *Ppa-ebax-1* destabilizes the largest miRNA cluster in *P. pacificus*, the *miR-2235a*/*miR-35* family. This destabilization suggests that the *miR-2235a* locus might undergo target-directed miRNA degradation (TDMD or TDMD-related processes). While miRNAs typically direct degradation of their mRNA targets, TDMD involve some targets with unusual miRNA-extensive pairing that direct degradation of cognate miRNAs. Our current model hypothesizes that an unknown trigger after bacterial dietary exposure induces *Ppa-ebax-1* activity leading to *miR-2235a* destabilization. As a consequence of miRNA degradation, target gene/s will be expressed resulting in TEI of the predatory morph. Consistent with this model, we showed that the most *ebax-1*-sensitive *miR-2235a* is a repressor of epigenetic memory, with the deletion of the entire cluster of more than 80 miRNA copies resulting in precocious inter- and transgenerational inheritance. Although TDMD is thought to regulate the levels of numerous miRNAs, mRNAs that endogenously direct miRNA degradation remain scarce in nematodes, but also flies and mammals.

By predicting miRNA binding sites across 3'UTR regions of *P. pacificus* genes, we identified hundreds of genes with pairing to the miRNA seed region but also extensive pairing to the miRNA. By prioritizing top RNA triggers having extensive pairing with the *miR-2235a* locus, here, we report RNA triggers necessary for the maintenance of TEI. By generating partial and complete deletions of the miRNA binding site in the 3'UTRs of these predicted RNA triggers, TEI of the predatory mouth-form is no longer observed. To further probe the molecular consequences of disrupting key components of TDMD processes, we used overexpression studies of RNA triggers. Finally, we synthetically added the miRNA binding site into RFP transgenes and into naturally *miR-2235a*-free 3'UTRs of *P. pacificus* genes to test *miR-2235a* function. Together, the ease of using the binary read-out of mouth-form plasticity allowed the identification of TDMD triggers in the transmission of epigenetic memory. This study supports the importance of TDMD processes in general, and their significance for the control of organismal traits in an ecological context.

163 Multi-dimensional regulation of *lin-28* expression in the *C. elegans* heterochronic network

Charles Nelson, Victor Ambros Program in Molecular Medicine, University of Massachusetts Chan Medical School

LIN-28 is a highly conserved RNA binding protein that regulates animal pluripotency and development. In *Caenorhabditis elegans*, *lin-28* is a component of the heterochronic (developmental timing) gene regulatory network, and loss-of-function mutations of *lin-28* result in precocious larval development. Previous studies have suggested that the downregulation of LIN-28 protein during larval development is due to decreases in mRNA abundance and translational repression presumably through 3' UTR sequences complementary to the *let-7*-family and *lin-4* microRNAs, and protein destabilization by the long non-coding RNA *lep-5*. To date, no study has examined the endogenous regulation imposed by these *let-7*-family and *lin-4* microRNA complementary sequences, and it is currently unknown if *let-7*-family and *lin-4* microRNA-mediated repression through the endogenous *lin-28* 3' UTR affects *lin-28* mRNA abundance and/or LIN-28 translational inhibition. Moreover, the combined contributions of microRNA-mediated repression of LIN-28 expression and *lep-5*-mediated destabilization of LIN-28 protein remain unclear.

Using CRISPR/Cas editing of the endogenous *lin-28* 3' UTR, we find that complementary sites to the *let-7*-family and *lin-4* microRNAs function semi-redundantly for repression of LIN-28 by regulating both mRNA abundance and inhibition of translation, and act in a synergistic-like manner with the post-translational repression of LIN-28 exerted by *lep-5*. Interestingly, we find that deletion of the entire *lin-28* 3' UTR results in animals with a near wild-type phenotype, indicating that the *lin-28* 3' UTR contains cis-acting elements that mediate positive regulation of LIN-28 expression and counteract the repressive activity of the *let-7*-family and *lin-4* microRNAs and *lep-5*. Through the generation of multiple *lin-28* 3' UTR truncations, we identify three positive regulatory regions that serve to promote LIN-28 expression through the stabilization of its mRNA.

Despite their wild-type appearance, we find that many of these 3' UTR mutants are sensitive to further perturbation of LIN-28 expression as well as to exposure to the pathogenic bacterium *Pseudomonas aeruginosa.* Our results demonstrate that these multiple layers of strict regulation of LIN-28 expression – mRNA stability, protein synthesis, and protein stability – ensure that LIN-28 is properly and precisely downregulated at the correct time regardless of environmental conditions. Moving forward, we aim to examine how the *let-7*-family and *lin-4* microRNA sites cooperate to downregulate LIN-28 expression, the mechanism of how the positive regulatory regions promote LIN-28 expression, and how these various layers of LIN-28 expression contribute to the developmental robustness of *C. elegans*.

164 DAF-16/FOXO maintains multipotency during dauer though regulation of the transcription factor NHR-23/ROR and inhibition of the *let-7* miRNA family

Himani Galagali[1,2], Amelia F Alessi[3], Matthew J Wirick[4], Margaret R Starostik[2], Priya Balamurugan[2], Shreya Singireddy[2], Ruhi Patel[5], Suhua Feng[5], Steven E Jacobsen[5], Alison R Frand[5], Xantha Karp[4], John K Kim[21]Molecular Biology, Massachusetts General Hospital, [2]Johns Hopkins, [3]Biology, Johns Hopkins, [4]Central Michigan University, [5]UCLA

A fundamental challenge in development is balancing growth with the ability to withstand environmental stress.In *C. elegans*, stress triggers L2 larvae to enter dauer, a stress-resistant, quiescent, and reversible state that preserves cellular multipotency. We identify key interactions between conserved transcription factors DAF-16/FOXO and NHR-23/ROR, along with the pro-development *let-7* miRNA family, that regulate the transition from development to dauer. Using *daf-7(e1372)* mutants, which constitutively enter dauer at 25°C, we previously found that during dauer, *daf-16* prevents epidermal cells from precociously adopting differentiated characteristics, including expression of the adult collagen, *col-19*. Adult cell fates and timing of *col-19*expression are regulated by the heterochronic gene network, including *lin-41*, a *let-7* target. We show that *lin-41* downregulation, in a 3'UTR-dependent manner, is required for the misexpression of a *col-19::gfp* transgene in *daf-16(0)* dauers, suggesting regulation by *let-7*. Further analysis reveals that *daf-16(0)* dauers have elevated levels of the *let-7* family. ChIP-seq data show that DAF-16 binds upstream of *nhr-23*, the transcriptional activator of *let-7*, and that *daf-16(0)* dauers exhibit increased *nhr-23* mRNA and protein levels. Notably, *nhr-23* levels are also upregulated in a *daf-16* DNA-binding dead mutant, suggesting that DAF-16 directly represses *nhr-23* expression to limit *let-7* family levels and maintain multipotency during dauer. Comparative analysis of DAF-16 ChIP-seq data and mRNA-seq in *daf-16(0)* dauers identified 681 genes potentially repressed by DAF-16, many of which encode pro-growth genes involved in mitotic DNA replication and translational elongation. Additionally, DAF-16 may regulate 59 transcription factors that could reprogram gene expression to sustain multipotency and establish quiescence during dauer. Supporting this, we find that the misexpression of molting cycle genes in *daf-16(0)* L2d larvae is suppressed upon depletion of NHR-23, a molting cycle-associated transcription factor. Together, our findings support a model in which DAF-16 plays a dual role in dauer: activating stress response pathways while repressing developmental gene programs. By repressing *let-7* expression through direct regulation of *nhr-23*, DAF-16 ensures the maintenance of multipotency and quiescence under stress conditions.

165 3' Nucleotide asymmetry directs miRNA strand selection

Jeffrey C Medley[1], Sumire Kurosu[1], Huiwu Ouyang[2], Heather Crawshaw[3], Sarah Zhang[3], Ganesh Panzade[4], Will Sydzyik[3], Joel Sydzyik[3], Mira Bhandari[3], Christopher Hammell[2], Anna Zinovyeva[31]Division of Biology, Kansas State University, [2]Cold Spring Harbor Laboratory, [3]Division of Biology, Kansas State University, [4]Frederick National Laboratory for Cancer Research

microRNAs (miRNAs) are central regulators of gene expression and are essential for animal development. During miRNA biogenesis, double stranded precursors are processed into ~22-nucleotide duplexes that comprise two functionally distinct strands. The guide strand is loaded into an Argonaute protein to form the miRNA-induced silencing complex (miRISC), while the passenger strand is degraded. As the silencing activity of the miRISC is directed at transcripts containing sequence complementarity to the miRNA guide strand, asymmetric strand choice effectively determines the target repertoire of the miRISC. While alternative miRNA strand selection occurs in certain developmental and tissual contexts, improper strand selection has also been reported in several human diseases including cancers. Previous studies have indicated that asymmetries within miRNA duplex ends determine miRNA strand choice. Argonaute prefers to load the duplex end containing a favorable 5' nucleotide (U>A>C>G) and lower thermodynamic stability *in vitro*. However, these guidelines cannot fully explain miRNA strand preference, indicating additional features influence miRNA strand selection *in vivo*. Here, we demonstrate a conserved role for 3' nucleotide asymmetry in facilitating miRNA strand selection. We show that a 3' cytosine on miRNA passenger strands is highly favorable for guide strand selection in *C. elegans*, whereas thermodynamic asymmetries are largely dispensable for proper strand choice. In otherwise symmetrical duplexes, mutating a 3' nucleotide to cytosine was sufficient to drive selection of the opposite strand. By expressing exogenous miRNA variants in human HEK293T cells, we also show that 3' nucleotide asymmetry plays an evolutionarily conserved role in miRNA strand selection. Interestingly, a 3' cytosine on passenger strands was less favorable in human cells, suggesting species-specific differences in 3' nucleotide preference. We propose that nucleotide asymmetries on both strands of miRNA duplexes promote accurate strand selection and that changes in terminal nucleotide identities may contribute to alternative strand choice. Collectively, our findings establish an improved model of how miRNA strand selection is determined *in vivo*. These results lay the foundation to better understand the link between miRNA dysregulation and human disease and may also improve the rational design of miRNA-based therapeutics that rely on accurate guide strand selection.

166 Investigating the composition of SIMR foci and germ granules using TurboID proximity labeling

Shihui Chen, Carolyn M Phillips Molecular Biology, University of Southern California

RNA interference (RNAi) is an evolutionarily conserved gene silencing process that protects genome integrity and regulates gene expression. In *C. elegans*, most of the RNAi components are organized into phase-separated germ granules at nuclei periphery, including P granule, *Mutator* foci, Z granule, SIMR foci, P bodies, E granule, and D granule. Each germ granule exhibits distinct functions, spatial-temporal expression patterns, and hierarchical assembly. However, their composition and roles remain incompletely understood. We focused on elucidating the function of SIMR foci by investigating its protein components. Previously, we identified core SIMR foci components, including HRDE-2 and ENRI-2, which are essential for small RNA loading specificity of nuclear Argonaute proteins in the germline and embryos of *C. elegans*.

To uncover additional SIMR foci components, we have utilized TurboID proximal labeling technique. By tagging SIMR-1 with TurboID, a biotin ligase, we successfully labeled SIMR-1 proximal proteins and captured 252 genes from Mass spectrometry. Next, we conducted an RNAi genetic screen and identified 26 proteins required for SIMR foci assembly. Additionally, we sought to identify novel granular localized proteins. We tagged 14 uncharacterized proteins using CRISPR and found that 7 of them localize to germ granules. We are currently generating mutants for these candidates and performing small RNA sequencing and additional phenotypic analysis to examine their roles in germ granule organization and small RNA biogenesis. Overall, the identification and characterization of these new germ granule-associated proteins will contribute to a deeper understanding of the composition and function of each germ granule compartment in *C. elegans*.

167 Muscle-Specific and Direct Transcriptional Targets of DAF-16/FOXO Activated by Reduced Insulin/IGF-1 Signaling

Shifei Wu[1], Yan Li[2], Erin Gao[1], Charline Roy[3], Ying Wang[2], Ben Mulcahy[2], William Li[2], Sruthy Ravivarma[4], Wesley L Hung[2], John A Calarco[5], Mei L Zhen[1,21]Molecular Genetics, University of Toronto, [2]Lunenfeld-Tanenbaum Research Institute, [3]Université de Lyon, [4]Physiology, University of Toronto, [5]Cell and System Biology, University of Toronto

C. elegans insulin/insulin-like growth factor 1 signaling (IIS) regulates diverse physiological processes through the DAF-16/FOXO transcription factor. Although DAF-16 is present in all somatic cells, its effects are highly tissue-specific and involve tissue cross-talk. This suggests that distinct, tissue-specific DAF-16 transcriptional programs contribute to the functional diversity of IIS. Several previous studies have identified DAF-16-regulated gene targets in different tissues.

Using fluorescence-activated cell sorting (FACS) of GFP labeled muscle cells from animals with varying levels of IIS/DAF-16 activity, we identified genes in muscle that are up-regulated or down-regulated in response to increased DAF-16 activity. To refine our analysis, we curated DAF-16 docking sites in muscle-, neuron-, and gut-specific genes using published chromatin immunoprecipitation sequencing (ChIP-seq) datasets. This combined approach revealed 14 transcripts as potential direct, muscle-specific DAF-16 targets whose expression is influenced by IIS/DAF-16 activity.

We validated the expression sites and DAF-16 dependency of several candidate genes using a combination of transcriptional and translational reporters, as well as CRISPR genome editing to tag endogenous loci. Among these candidates, we confirmed the following genes as direct DAF-16 targets with strong and specific muscle expression: C54F6.5 (encoding a putative secreted protein), MLCD-1 (a metabolic enzyme), and CEX-1 (a calcium-binding protein previously thought to be expressed only in a pair of interneurons). Notably, these genes exhibit DAF-16-independent expression in non-muscle cells, which likely explains their low rank or absence from whole-animal microarray or mRNA-seq analyses of DAF-16 targets.

Our findings support the existence of tissue-specific DAF-16 transcriptional programs and underscore the importance of examining FOXO targets in a cell-type-specific manner.

168 Argonaute-siRNA loading via the RNA binding protein RDE-4

Thiago Leite Knittel[1], Brooke E. Montgomery[1], Reese A. Sprister[1], Colin N. Magelky[1], Margaret J. Smith[1], Maritza Soto-Ojeda[1], Carolyn M. Phillips[2], Tai A. Montgomery[11]Colorado State University, [2]University of Southern California

Small regulatory RNAs associate with Argonaute proteins to control gene expression, impacting a wide range of cellular processes, such as antiviral defense, transposon silencing, and development. Plants and animals typically have several classes of small RNAs, along with multiple Argonautes. These Argonaute often confer distinct functionality to the various classes of small RNAs. But how the different small RNAs are matched with the appropriate Argonaute is not well understood. Here, we show that the *C. elegans* double-stranded RNA binding protein RDE-4 facilitates the loading of small interfering RNAs (siRNA) into the Argonaute RDE-1, but not ALG-1, and loading of 26G-RNAs into the Argonaute ERGO-1, but not ALG-3 and ALG-4. Nonetheless ALG-3/ALG-4 class 26G-RNAs are depleted in *rde-4* mutants, consistent with the previously described role of RDE-4 in Dicer processing of double-stranded RNA. Our results reveal a key factor in small RNA loading specificity and help to explain how certain small RNAs are matched with specific Argonautes in C. elegans.

169 Genetically distinct silencing pathways monitor 5' and 3' splicing site defects in *C.elegans*

Min Li, Sara Keane, Masaki Shirayama, Craig Mello UMass Chan Medical School

In the *C. elegans* germ line, genes lacking introns or containing long exons undergo stable, transgenerational silencing via a heterochromatin/small RNA pathway that shares downstream components with epigenetic silencing triggered by piRNAs and dsRNAs. However, the mechanisms by which worms detect defective splicing remain unclear. In humans, the human silencing hub (HUSH) complex silences long, intron-less transgenes, including LINE-1 retrotransposons, by depositing repressive histone modifications. We recently identified TASR-1 and the chromodomain protein CEC-10, worm homologs of HUSH components, as required for intron-less gene silencing, suggesting a conserved splicing-sensitive epigenetic pathway.

To dissect the triggers of intron-less silencing, we developed a reporter assay inserting *gfp* with or without functional introns into the endogenous *his-61* locus. In wild-type animals, intron-less *gfp* was silenced in a *tasr-1*-dependent manner. Furthermore, *tasr-1* was required solely to trigger silencing; once established, the loss of *tasr-1* did not reverse the silencing. Deep sequencing and transitive silencing assays revealed that small RNAs were generated in intron-less *gfp::his-61* animals, and these small RNAs were capable of silencing an intron-containing reporter, *gfp::cdk-1*, in trans.

To determine whether splicing defects alone could trigger transitive silencing, we introduced *gfp* with mutations at either the 5' or 3' splice sites. Strikingly, a single mutation at either splice site was sufficient to cause transitive silencing. However, the 5› splice site mutation specifically required TASR-1 for silencing, while the 3› splice site mutation led to TASR-1-independent gene silencing. These findings reveal the existence of two distinct pathways for detecting splicing defects in *C. elegans*.

In conclusion, we have identified an additional layer of genome surveillance that represses genes with defective pre-mRNAs in the nucleus. This mechanism complements the existing piRNA and dsRNA pathways, which protect the germline from unlicensed genetic elements.

170 A lncRNA degrades an embryonic microRNA family in a noncanonical mechanism

Acadia Grimme[1,2], Lu Li[2,3], Mingyie Xie[3], Katherine McJunkin[41]National Institute of Diabetes and Digestive and Kidney Diseases, [2]Johns Hopkins University, [3]University of Florida, [4]Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases

MicroRNAs (miRNA) are small RNAs that are loaded into an Argonaute protein to form a complex that post-transcriptionally regulates gene expression. miRNA abundance is controlled at both the level of biogenesis and degradation. One such degradation mechanism is known as target-directed miRNA degradation (TDMD). In canonical TDMD, extensive interactions between the miRNA and a decay-triggering RNA allow for the TDMD effector protein ZSWIM8 to ubiquitylate the Argonaute, leading to degradation of the protein and miRNA. In *C. elegans*, we find that the *mir-35* miRNA family seed sequence is sufficient for developmentally-timed miRNA degradation dependent on the ZSWIM8 ortholog EBAX-1; this therefore represents a new mode of TDMD mediated by seed interactions rather than extensive base-pairing. The Argonaute proteins ALG-1 and ALG-2 are both sensitive to seed-mediated TDMD in late-stage embryos. We used CLASH to identify the long non-coding RNA *tts-2* as the RNA trigger of *mir-35* family TDMD. Deletion of *tts-2* results in the stabilization of all eight of the *mir-35* family members. Through additional extensive CRISPR editing of *tts-2*, we find that *tts-2* drives *mir-35* family degradation through seed-mediated TDMD. These results demonstrate that TDMD can occur via multiple RNA-RNA architectures outside of the canonical extensive pairing and opens the possibility of targeting entire miRNA families through seed-mediated TDMD for future therapeutic applications.

171 Proximity Labelling at H3K9me3 Reveals VRK-1 as a Candidate Anti-Silencing Factor

William Smith[1], Valeryia Aksianiuk[1], Devanarayanan Siva Sankar[2], Joern Dengjel[3], Rodrigo Villaseñor[4], Tuncay Baubec[5], Peter Meister[61]Institute of Cell Biology, University of Bern, [2]Global Health Institute, EPFL, [3]Department of Biology, University of Fribourg, [4]Molecular Biology Division, Biomedical Center Munich, [5]Division of Genome Biology and Epigenetics, University of Utrecht, [6]Cell Biology Institute, University of Bern

A key mechanism regulating chromatin states is the post-translational modification (PTM) of histones, with H3K9me3 marking transcriptionally repressed heterochromatin. Repressive histone PTMs recruit remodelling factors that maintain heterochromatin or promote decompaction, initiating euchromatin formation. We developed a proximity labelling technique to identify proteins recruited to H3K9me3, reproducibly identifying many known H3K9me3 binders and ~150 novel factors, including the chromatin-associated kinase VRK-1. Notably in humans, VRK1 loss increases repressive histone marks, including H3K9me3, while reducing active marks, suggesting it has roles in negatively regulating H3K9me3. Using quantitative microscopy, we revealed that stress, including heat shock, enriches VRK-1 at the nuclear periphery, where H3K9me3 is concentrated. Heat shock also induces a reversible large-scale chromatin shift toward the periphery and chromatin condensation. We hypothesised that VRK-1 regulates these stress-induced changes, and indeed, VRK-1 depletion greatly reduces the removal of peripheral chromatin during heat shock recovery. BAF-1, a known VRK-1 substrate, mediates chromatin condensation and nuclear periphery anchoring, with VRK-1 phosphorylation reducing its chromatin affinity. RNAi knock-down of *baf-1* in a VRK-1 depleted background improves chromatin recovery after heat shock, suggesting VRK-1 loss leads to excessive BAF-1 retention, promoting hyper-compaction and periphery attachment. We next aim to examine VRK-1's role in gene expression under normal and stress conditions as well as the genome-wide distribution of VRK-1. Together, our findings suggest that VRK-1 facilitates chromatin decompaction, possibly in a BAF-1-dependent manner, revealing a potential new regulatory mechanism.

172 A *C. elegans* model of *PNPT1*-dependent hereditary hearing loss

Xu Bai[1], Yuling Guo[1], Can Wang[1], Jianfeng Liu[2], X.Z. Shawn Xu[11]Life Sciences Institute, University of Michigan, [2]College of Life Science and Technology, Huazhong University of Science and Technology

Hearing was thought to exist only in vertebrates and some arthropods. Strikingly, our recent research revealed that *C. elegans* can sense airborne sound through the sound-sensitive FLP and PVD neurons and engage in phonotaxis behavior. In a genetic screen for mutants defective in auditory sensation, we identified a mutant strain of *pnpt-1*, which encodes polynucleotide phosphorylase (PNase or PNPT1) important for mitochondrial RNA import and processing. This mutant showed a specific defect in auditory sensation, while its other sensory functions remained largely normal. Interestingly, *PNPT1*, the human ortholog of *C. elegans pnpt-1*, has been reported as a deafness gene, and mutations in this gene cause non-syndromic hearing loss without affecting other sensory functions via an unknown mechanism. However, no animal model is currently available to characterize *PNPT1* in auditory sensation. To dissect the underlying mechanisms, we introduced the deafness-causing mutation E475L in *PNPT1* of human patients into *C. elegans pnpt-1* by CRSIPR/Cas9 genome editing. Remarkably, the knockin mutant worm also exhibited a specific defect in auditory sensation, recapitulating the symptom found in human patients. This suggests a functional conservation of *pnpt-1*/*PNPT1* from invertebrates to vertebrates. As *pnpt-1*/*PNPT1* is expressed in all cell types, the question arises as to how mutations in *pnpt-1*/*PNPT1*, a ubiquitously expressed gene, could specifically affect the auditory system. We found that *pnpt-1* mutant worms exhibit functional deficits in mitochondria, including decreased ATP and increased ROS levels. Importantly, like hair cells in human inner ear, the sound-sensitive FLP/PVD neurons also possess a higher abundance of mitochondria compared to other sensory neurons, indicating a high demand for energy. These data suggest a model that sound-sensitive neurons are more vulnerable to mitochondrial dysfunction, revealing how non-syndromic mutations in *pnpt-1*/*PNPT1* could cause a deficit in the auditory but not in other sensory systems. Our findings establish *C. elegans* as a valuable model for studying *PNPT1*-dependent hereditary hearing loss.

173 Presynaptic adaptation of the co-transmitter AFD thermosensory neuron modulates navigation across a thermal gradient

Andrea A Cuentas Condori[1], Patricia Chanaba Lopez[1], Malcom Diaz Garcia[1], Joon Lee[1], Daniel Colon-Ramos[1,2,31]Neuroscience, Yale University, [2]Wu Tsai Institute, [3]Instituto de Neurobiologia, Universidad de Puerto Rico

Changes in the presynaptic machinery encode animal behavior. In neurons that use more than one neurotransmitter, it is critical to understand if there are transmitter-specific adaptations that result in changes in behavior. To visualize co-transmitter synapses *in vivo*, we developed new genetic tools to label all transmitter-specific synaptic vesicle transporters at the endogenous level and with single-cell resolution. Now, we can monitor transmitter-specific presynaptic machinery in any synapse. Using these tools, and the latest neurotransmitter atlas, we find that 10% of *C. elegans* neurons have co-transmission potential, most of which are sensory neurons.

In the thermosensory neuron AFD, co-transmission of glutamate and acetylcholine regulates navigation to a preferred temperature. AFD encodes the thermal preference of *C. elegans*; where animals learn to prefer a temperature based on their experience. We found that the Vesicular Glutamate Transporter, EAT-4, increases with warmer temperature, while the Vesicular Acetylcholine Transporter, UNC-17, decreases with warmer temperature. This presynaptic adaptation happens only a few hours after the animal experiences a new temperature. Consistent with the idea that co-transmission of glutamate and acetylcholine modulate thermotaxis, *tax-4* mutants that are athermotactic result in the decrease of EAT-4 levels and the complete loss of UNC-17 in AFD neurons.

To understand how these neurotransmitters may modulate thermotaxis behavior *in vivo*, we took advantage of the AFD-specific constitutive activation of PKC-1 (caPKC-1), which results in cryophilic animals. In caPKC-1 animals, AFD-specific disruption of glutamatergic signaling prevents cryophilic behavior, while disrupting the cholinergic signaling seems to improve it. Interestingly, when looking at the presynaptic machinery of caPKC-1 animals, EAT-4 and UNC-17 levels always resemble those found in animals grown at colder temperatures. Thus, the conserved presynaptic modulator PKC-1 seems to lock the presynaptic machinery to mimic that of animals grown at colder temperatures. Overall, our findings show that transmitter-specific adaptations of the presynaptic machinery of AFD are required to modulate the thermal preference of an animal. Because sensory systems from nematodes to mammals have co-transmission capacity, our studies are shedding light on the molecular mechanisms that remodel presynaptic terminals in transmitter-specific manners to control behavior.

174 Interspecies relationships and microbial ensembles shape Dauer formation in natural and extreme environments

Marcela Serey, Esteban Retamales, Gabriel Ibañez, Juan Pablo Castillo, Andrea Calixto Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso

Microbial interactions shape ecological stability, yet how bacteria, amoebae, and nematodes influence each other's behaviors remains poorly understood. Dauer formation is a key nematode survival strategy, but its natural triggers remain largely unknown. In this study, we developed a framework to examine long-term interactions between microbes and animals in both temperate and extreme environments. From soil samples collected in a temperate, semi-arid climate, we isolated *Comamonas, Stenotrophomonas, Chryseobacterium*, and *Rhodococcus*, as well as the amoeba *Tetramitus*. This microbial ensemble, fed to *C. elegans* for over 20 generations, induced Dauer Formation on Naturally derived Ensembles (DaFNE)—a phenomenon in which nematodes enter diapause after multiple generations in a microbially stable environment.

DaFNE intensifies over time and depends on both the nematode's pheromone biosynthesis and RNA interference (RNAi) pathways. We show that mutations in *sid-2*, essential for dsRNA entry into intestinal cells, and *rde-1*, required for processing of exogenous dsRNA, abolish DaFNE. Notably, DaFNE displays a transgenerational memory effect, in which dauer formation is accelerated upon re-exposure to the microbial ensemble after a two-generation pause. This effect requires *HRDE-1* and *ZNFX-1*, further supporting a role for small RNA pathways in bacterially induced behavioral adaptation across generations.

We next explored soil microbiomes from the Atacama Desert, one of the driest and most oligotrophic places on Earth. We found that bacterial communities enriched in vitamin B12 producers correlate with the presence of nematodes, suggesting that microbially derived metabolic cues contribute to dauer formation in extreme settings, paralleling DaFNE. To further explore these microbial influences, we tested the ability of *C. elegans* to enter diapause under high temperatures. We found that vitamin B12-producing bacteria, as well as B12 supplementation alone, significantly increased dauer penetrance at 27°C, even in the presence of *E. coli* OP50. This suggests that specific bacterial metabolites act as ecological signals influencing nematode persistence and dauer formation in harsh environments.Together, our findings highlight how microbial metabolites shape nematode developmental trajectories across diverse ecological contexts, revealing conserved interspecies interactions that drive transgenerational behavioral adaptation and survival strategies.

175 Nictation: Neurons, Genes, and Connectome

Junho Lee Seoul National University

Since 1960s, *C. elegans* has been served as a model organism for development and the nervous system. We have been studying nictation, the hitch-hiking behavior specific to dauer, an alternative developmental stage of nematodes with distinct behavioral characteristics. We have shown that IL2 ciliated sensory neurons are required for nictation. An isoform of DAF-19, DAF-19M, was required for differentiating IL2 neurons from other ciliated neurons for nictation. GWAS analyses allowed us to identify genes that regulate nictation, in particular, glial expression of a steroidogenic enzyme was necessary for regulating nictation in wild isolates. To examine the developmental plasticity of the nervous system in dauer, we reconstructed the complete chemical connectome of dauer by volumetric reconstruction and automated synapse detection using deep learning. With the basic architecture of the nervous system preserved, structural changes in neurons, large or small, were closely associated with connectivity changes, which in turn evoked dauer-specific behaviors such as nictation. Graph theoretical analyses revealed higher clustering of motor neurons and rewiring of sensory outbound connections in the dauer connectome. We suggest that the nervous system in the nematode, probably animals in general, has evolved to respond to harsh environments by reversibly developing a quantitatively and qualitatively differentiated connectome.

176 SRS microscopy identifies inhibition of vitellogenesis as a mediator of lifespan extension by caloric restriction in *C. elegans*

Bowen Yang[1], Bryce Manifold[2], Wuji Han[3], Catherin DeSousa[1], Wanyi Zhu[1], Aaron Streets[2,3,4], Denis Titov[1,3,5]1Nutritional Science & Toxicology, UC Berkeley, [2]Bioengineering, UC Berkeley, [3]Computational Biology, UC Berkeley, [4]Chan Zuckerberg Biohub, [5]Molecular and Cell Biology, UC Berkeley

The molecular mechanisms of aging are not fully understood. Here, we used label-free Stimulated Raman scattering (SRS) microscopy to investigate changes in proteins and lipids throughout the lifespan of *C. elegans*. We observed a dramatic buildup of proteins within the body cavity or pseudocoelom of aged adults that was blunted by interventions that extend lifespan: caloric restriction (CR) and the reduced insulin/insulin-like growth factor signaling (IIS) pathway. Using a combination of microscopy, proteomic analysis, and validation with mutant strains, we identified vitellogenins as the key molecular components of the protein buildup in the pseudocoelom. Vitellogenins shuttle nutrients from intestine to embryos and are homologous to human apolipoprotein B, the causal driver of cardiovascular disease. We then showed that CR and knockdown of vitellogenins both extend lifespan by >60%, but their combination has no additional effect on lifespan, suggesting that CR extends the lifespan of *C. elegans* in part by inhibiting vitellogenesis. The extensive dataset of more than 12,000 images stitched into over 350 whole-animal SRS images of *C. elegans* at different ages and subjected to different longevity intervention will be a valuable resource for researchers interested in aging.

177 The SPN-4 RNA-binding protein promotes maternal mRNA clearance during the oocyte-to-embryo transition

Erin Osborne Nishimura[1], Caroline Spike*[2], Dylan Parker*[3,4], Tatsuya Tsukamoto*[2], Naly Torres*[5], Micah Gearhart[6], Erika Tsukamoto[2], Karissa Coleman[1], David Greenstein†[71]Biochemistry & Molecular Biology, Colorado State University, [2]Genetics, Cell Biology & Development, University of Minnesota, [3]Department of Biochemistry, University of Colorado Boulder, [4]Howard Hughes Medical Institute, University of Colorado Boulder, [5]Cell and Molecular Biology, Colorado State University, [6]Genetics, Cell Biology and Development, University of Minnesota, [7]Genetics, Cell Biology, & Development, University of Minnesota

*Contributed equally to this work †Corresponding authors

In many species, maternal mRNAs are cleared after they have fulfilled their functions. We have uncovered an essential pathway that clears maternal mRNAs from *C. elegans* embryos using the SPN-4 cytoplasmic Rbfox-related RNA-binding protein and the Ccr4-Not deadenylase complex. The oocyte translational regulators LIN-41 and OMA-1/2 mediate a mutually antagonistic repression-to-activation switch that triggers SPN-4 expression at the end of oogenesis. We identified 728 mRNAs that associate with SPN-4 in late-stage oocytes by conducting immunopurification of SPN-4 followed by RNA-seq on captured nucleic acids. Many SPN-4-associated mRNAs decay following fertilization. Indeed, using single-molecule FISH, we found that *spn-4* depletion leads to an over-abundance of its associated mRNAs. Two sequences matching consensus Rbfox protein binding sites in the 3'UTRs of two SPN-4 targets (*lin-41* and *chs-1* mRNA) are required for SPN-4 dependent clearance. We therefore propose that the *spn-4* maternal-effect lethal phenotype results from an inappropriate retention of maternal mRNAs through the oocyte-to-embryo transition.

Genetic studies of the Pumilio-related RNA-binding proteins, PUF-3 and PUF-11, led us to identify the involvement of Ccr4-Not complex in SPN-4-dependent mRNA clearance. SPN-4 is prematurely expressed in *puf-3/11* double null oocytes, suggesting that premature mRNA degradation might cause the *puf-3/11* embryonic lethal phenotype. Consistent with this, we isolated mutant alleles of SPN-4 and Ccr4-Not complex genes in a large-scale genetic screen for dominant suppressors of the *puf-3/11* lethal phenotype. Likewise, auxin-induced degradation of key Ccr4-Not components (LET-711/Not1 and CCF-1) disrupts SPN-4 associated mRNA clearance. This suggests that SPN-4 utilizes the Ccr4-Not complex to target its associated mRNAs for degradation during the oocyte-to-embryo transition. Our results suggest a model in which SPN-4 initiates expression in late-stage oocytes, associates with its maternal mRNA targets by binding their 3'UTRs, and then recruits the Ccr4-Not complex to promote mRNA decay. This mechanism contributes to the widespread changes that occur in the oocyte-to-embryo transition

178 Dynamic regulation of the proteasome by ECPS-1/Ecm29.

Sheikh Omar Kunjo, Irini Topalidou, Sarah Tomlin, Nicolas Lehrbach Basic Sciences Division, Fred Hutchinson Cancer Center

The proteasome is essential for most cellular functions and enforces protein quality control by destroying damaged and misfolded proteins. Impaired proteasome function is a cause of proteostasis failure in aging and neurodegenerative diseases, whereas excessive protein degradation is a feature of cancers and autoimmune disorders. Thus, precise regulation of proteasome levels and activity are required to ensure homeostasis and allow adaptation to stress. Proteasomes associate with a plethora of proteasome-binding proteins that may serve to fine-tune protein turnover. The physiological significance of these interactions, and how they are regulated in different contexts, is poorly understood. Here, we reveal dynamic regulation of proteasome function and proteotoxic stress resistance by the evolutionarily conserved proteasome-binding protein ECPS-1/Ecm29. We identified loss of function mutations affecting *ecps-1* in a screen for suppression of infertility caused by a proteasome subunit mutation affecting *rpn-5* (a component of the 19S regulatory particle). Surprisingly, we find that *rpn-5* mutants are resistant to pharmacological proteasome inhibition, but inactivation of *ecps-1* reverses this resistance. Loss of ECPS-1 increases wild type animals' sensitivity to proteasome inhibitor drugs, suggesting an *ecps-1*-dependent pathway that safeguards proteasome function might be hyperactivated in the *rpn-5* mutants. To better understand the mechanism by which ECPS-1 is regulated, we examined the subcellular localization of GFP-tagged ECPS-1. ECPS-1 is found in both the nucleus and cytosol, but it becomes enriched in nuclei after proteasome inhibition. In contrast, ECPS-1 is constitutively enriched in the nuclei of *rpn-5* mutant animals regardless of proteasome inhibition, consistent with resistance via hyperactivation of this response. We find that stress-responsive nuclear enrichment of ECPS-1 requires nuclear proteasomes and is accompanied by a rapid increase in physical association between ECPS-1 and both nuclear and cytosolic proteasomes. Remarkably, we detect increased binding of ECPS-1 to proteasomes in animals exposed to proteasome inhibitor for only 30 minutes, indicating rapid induction of this stress response. Collectively, these data suggest that ECPS-1/Ecm29 initiates an acutely regulated proteasomal stress response by selective binding to inhibited or dysfunctional proteasomes. Interestingly, we also find that *ecps-1* is required to maintain proteostasis in aging animals, suggesting that this dynamically regulated pathway may also promote longevity. We propose that ECPS-1/Ecm29 is a physiologically critical and dynamic proteasome regulator required to ensure homeostasis and allow cellular adaptation to stress. As such, human Ecm29 may be a target for therapeutic manipulation in diseases associated with proteasome misregulation.

179 The *C. elegans* Connectome Toolbox: consolidating and analyzing datasets of multimodal connectivity for *C. elegans*

Yasinthan Vickneswaran[1], Stephen D Larson[2,3], Ankur Sinha[1], Padraig Gleeson[1]1Neuroscience, Physiology and Pharmacology, University College London, [2]OpenWorm Foundation, [3]MetaCell

The nematode *C. elegans* is one of the best studied model organisms in biology. Numerous published studies have quantified and analyzed the chemical and electrical synaptic connections between the neurons in the worm since the first description of the connectome by White and colleagues, and many of these studies have released structured datasets of this connectivity. These datasets have formed the basis of many subsequent studies into the neuronal basis of *C. elegans* behavior. However, there is no one fixed «*C. elegans* connectome», and these datasets have evolved over time, merging data from previous studies, adding new connections and using different interpretations of connection weights. In recent years, datasets have also become available for multiple developmental stages of the worm, as well as on the extrasynaptic connectivity of neurons (monoaminergic and peptidergic), and the functional connectivity between pairs of identified neurons.

We have reviewed the historical data on *C. elegans* connectomics, and have created a single, user-friendly Python based software package offering uniform programmatic access to the data, along with an online resource for easy navigation of these multimodal connectome datasets (https://openworm.org/ConnectomeToolbox), providing multiple interactive views of the data. It is designed to be an extensible resource for the community, to which more datasets can be added as they are acquired. We will demonstrate how these assembled datasets can be used to examine how the bilateral symmetry of neurons varies for different synapse types, as well as across developmental stages of the worm. Additionally, we show how neuronal connectivity data generated from computational models of the worm can be integrated, illustrating how closely or otherwise the circuitry of the models align with published connectomic datasets.

This work is part of the OpenWorm project, a global, online collaboration of computational and experimental neuroscientists, software developers and interested volunteers with an ambitious long-term goal: creating a cell-by-cell computer model of *C. elegans* which reproduces the behavior of the real animal in as much detail as possible.

180 A Molecular Balancing Act: X-Chromosome Dosage Compensation

Rajarshi P Ghosh[1], Connor D Luellen[1], Yuri Malina[1], Dhruv Sidhwani[1], Miki Okada[1], Zoe Yeh[1], Bret Unger[2], Ke Xu[2], Barbara J Meyer[1]1Molecular & Cell Biology, HHMI / UC Berkeley, [2]Chemistry, UC Berkeley

The *C. elegans* dosage compensation complex (DCC) balances X gene expression between sexes by halving transcription from both hermaphrodite X chromosomes. Failure to do so kills hermaphrodites. We show stepwise assembly of DCC across X and how it impacts transcription.

Previously, we found that combinatorial clustering of DNA motifs at recruitment elements on X (*rex* sites) drives DCC recruitment. We now show that local chromatin accessibility is a critical recruitment feature. Once bound, the DCC repositions flanking nucleosomes.

STORM imaging shows that SDC-2, the pivotal DCC loader, forms uniform clusters on X. In contrast, DPY-27, a DCC-specific condensin subunit, forms a diffuse cloud around SDC-2 hubs. Hence, while SDC-2 initiates assembly, DPY-27 extends beyond nucleation sites via controlled dispersion dependent on its capacity to hydrolyze ATP.

The number of SDC-2 molecules inside clusters is invariant across protein concentrations, but cluster number scales with concentration, suggesting scaffold-limited clustering. To test whether *rex* sites act as nucleation scaffolds, we imaged *rex* sites using MINFLUX and STORM, and quantified *rex-rex* interaction probability at nucleosome resolution with Micro-C XL. A subset of *rex* sites showed spatial coalescence, suggesting a role in scaffold-limited clustering of SDC-2. Unlike SDC-2, which forms stable hubs, DPY-27 transits rapidly between hubs, assembling into nanodomains extending beyond edges of X chromatin, implying DPY-27 forms a semi-constrained, ATP-dependent network that fluidly bridges chromatin territories. Optodroplet assays show DCC subunits self-associate.

STORM and SIM imaging show partial exclusion of RNA Pol II from X chromatin, consistent with reduced transcription. We are exploring whether RNA Pol II is immiscible with DCC condensates.

Using a 3D Convolutional Neural Network for analyzing high-throughput fluorescent images of X-bound SDC-2 and DPY-27, and entropy-based similarity analysis, we reconstructed the path of DCC assembly.

Using Micro-C XL and GRO-seq, we assessed impact of DCC-driven chromatin remodeling on transcription at subgenic resolution. X is organized into distinct, hierarchically nested domains, with chromatin architecture tightly linked to transcription. DCC disruption collapses this organization, reversing domain configurations coincident with transcriptional shifts, underscoring interplay between DCC function, local chromatin topology and transcription.

Molecular dynamics simulations show that fine-tuned DCC concentration, cooperative self-association, and *rex* clustering drive X-chromatin restructuring, linking molecule-scale interactions to genome reorganization.

181A A hybrid non-motile Cilium requires motility apparatus for mechanosensation

Dhruvin Akbari[1,2], David Logan[3], Laura Bianchi[3], Michel Leroux[11]Molecular Biology and Biochemistry, Simon Fraser University, [2]Centre for Cell biology, Development, and Disease, Simon Fraser University, [3]Department of Physiology and Biophysics, University of Miami

The sensation of touch is experienced by all life. Almost without exception, the mechanosensory stimulus leads to rapid signal transduction by channel proteins. Sensory (non-motile) cilia are often employed to capture sensitive environmental cues, including physical force. The nematode *C. elegans* uses its sense of touch to navigate, forage, and evade predators by relying in part on its sensory cilia. Although *C. elegans* is the only metazoan to have lost the capacity to produce motile cilia, we discovered using the CeNGEN single-cell expression database transcripts coding for a few cilium motility proteins (CMPs). Remarkably, these are strictly found in the mechanosensory OLQ neurons. The genes include two dynein heavy chains (*dhc-3*, *dhc-4*), dynein intermediate (*dnai-7*) and light intermediate chains (*dyla-1*), an anchoring protein (*cfap-57*), and a radial spoke component (*nme-7*). The cilia of OLQ neurons harbor at least three known TRP-family of mechanosensory channels (TRPA-1, OSM-9 and OCR-4). We hypothesize that CMPs might facilitate touch sensation through functional interactions with the TRP channel proteins. We confirmed that all GFP-tagged CMPs localize to the axoneme of OLQ cilia tagging. Additionally, DYLA-1 depends on both DHC-3 and DHC-4 for its localization to OLQ cilia, consistent with their association as a complex in motile cilia. We showed that CMP mutants are defective in nose touch avoidance compared to wild-type animals, revealing for the first time a role for these motile cilia proteins in mechanosensation. Experiments are underway to uncover the nature of association between TRP channels and CMPs. Our study unveils a novel mechanism for touch sensation in non-motile cilia that relies on motile cilia proteins. Given that OLQ cilia harbor a functional complex of CMPs despite being a non-motile cilium, we term such cilia as hybrid. These surprising and seemingly unconventional combination of motility proteins in non-motile cilia hint at novel and complex mechanisms of touch sensation in cilia in vertebrates and other metazoans.

182A Using a kinesin-1 auxin-inducible degron to investigate the roles of kinesin-1 in locomotion and dense core vesicle transport in *C. elegans*

Astrid Boström, Anna Gavrilova, Gino Poulin, Viki Allan School of Biological Sciences, University of Manchester

Intracellular transport of organelles along microtubules is carried out by opposing anterograde- and retrograde-directed motor proteins that often bind to the same cargoes. The regulation of bidirectional cargo transport and the effects of the disruption of single motors is not fully understood. In humans, mutations in the anterograde motor kinesin-1 are associated with a number of neurological diseases, including ALS, CMT, and HSP, all characterised by movement defects. *Caenorhabditis elegans* has one gene for kinesin-1 heavy chain called *unc-116.* Complete loss of *unc-116* leads to embryonic lethality. Consequently, studies often rely on mutants with reduced motor activity, but these animals suffer from systemic defects from birth. We have therefore developed a conditional knockdown UNC-116 worm based on the Auxin Inducible Degron (AID) system, allowing UNC-116 degradation after larval development by the addition of auxin for controlled periods of time. Through tissue-specific expression of the F-box protein TIR1, we show that degrading UNC-116 either pan-somatically or pan-neuronally leads to an *uncoordinated* phenotype over time, opening for the possibility to use this system as a disease model. We also demonstrate that bidirectional transport of dense core vesicles (DCVs) in a *C. elegans* neuron is rapidly affected by UNC-116 degradation, supporting previous evidence of an important role of kinesin-1 in DCV transport and highlighting a role in controlling the balance between anterograde and retrograde transport. Together, these results indicate that the degron-UNC-116 worm is a valuable system for studying bidirectional transport regulation as well as systemic effects of kinesin-1 loss.

183A Nuclear vesicle release during neuronal extrusion events

Rebecca J Androwski[1], Ken Nguyen[2], David H Hall[2], Barth D Grant[1], Monica Driscoll[31]Molecular Biology and Biochemistry, Rutgers University, [2]Albert Einstein College of Medicine, [3]Rutgers University

A growing body of research highlights the vulnerability of neuronal nuclei in Alzheimer's disease (AD). AD brains exhibit increased DNA fragmentation, oxidative DNA damage, and abnormal accumulation of nuclear-localized proteins compared to non-disease controls, implicating nuclear dysfunction as a critical factor in neurodegeneration. Despite this, mechanisms of nuclear quality control, particularly in postmitotic, long-lived neurons under stress or during aging, remain poorly understood.

Using *C. elegans*, a powerful model for studying cellular resilience and aging, we have identified a novel mechanism of nuclear material management through nuclear vesicle (NV) extrusion. Previous work in the Driscoll Lab characterized the exopher, a large (~5 µm) vesicle that enables neurons to expel toxic aggregates and damaged organelles under stress. Exopher-like structures have been identified in *C. elegans*, rodent, and human systems, with increased numbers observed in AD brains. These findings suggest a conserved, neuroprotective role for exopher production in maintaining neuronal health during aging.

Unexpectedly, we discovered that *C. elegans* neurons under proteostress associated with a high copy number mCherry transgene extrude NVs that bud directly from the intact nucleus and are transported into the exopher for remote degradation by a neighboring cell. These NVs are surrounded by the inner nuclear membrane protein EMR-1 and components of the endoplasmic reticulum (which is contiguous with the nuclear outer membrane) but exclude the nucleolus. DAPI staining revealed that 53% of NVs contain detectable DNA, and serial electron microscopy (EM) sections show exopher cargo consistent with condensed heterochromatin. This selective packaging suggests that NV extrusion may represent a regulated process for removing damaged or unwanted nuclear material, potentially contributing to genomic stability and proteostasis in aging neurons. The identity of the nucleic acids extruded is a looming question I am pursuing.

Our findings provide a novel perspective on nuclear quality control mechanisms and their intersection with neuronal waste disposal. By characterizing NV formation and its functional role in proteostressed neurons, this research establishes a new avenue for exploring nuclear maintenance in aging and neurodegenerative disease contexts.

184A Germline regulation of the intestinal mitochondrial unfolded protein response

Anna Foulger[1], Suzanne Angeli[2], Dipa Bhaumik[1], Julie Andersen[1], Gordon Lithgow[11]Buck Institute for Research on Aging, [2]Molecular and Biomedical Sciences, University of Maine

The disposable soma theory posits that there is a trade-off between reproduction and somatic maintenance. In support of this theory, we previously identified that pharmacological inhibition of the germline has widespread protective cell non-autonomous effects on cellular protein homeostasis in *Caenorhabditis elegans*. Here, we use pharmacological or genetic inhibition of the germline to determine its effects on intestinal mitochondrial protein homeostasis as measured by the mitochondrial unfolded protein response (UPRmt). We find that pharmacological inhibition of germline proliferation by 5-fluoro-2-deoxyuridine (FUdR), a DNA synthesis inhibitor, potently inhibits activation of the intestinal UPRmt as well as reverses lifespan effects induced by loss of OXPHOS subunits. We find similar results with the genetic mutant (*glp-1*), which lacks germline proliferation. To further identify the reproductive processes required to regulate the intestinal UPRmt, we examined the genetic mutant *fem-1*, which contains an intact gonad but lacks sperm. Like *glp-1* mutants, *fem-1* mutants do not activate the intestinal UPRmt due to loss of OXPHOS subunits. Restoring reproduction in *fem-1* mutants by mating them with wild type males is sufficient to reactivate the intestinal UPRmt. Furthermore, loss of the FOXO transcription factor *daf-16* is also sufficient to reactivate the intestinal UPRmt in *fem-1* mutants and partially in *glp-1* mutants. These findings suggest that FOXO/*daf-16* acts to limit UPRmt activation in the intestine. These findings also suggest that late-stage reproductive signals from sperm and/or fertilization play a critical role in cell non-autonomous intestinal UPRmt activation.

185A Inward transport of organelles drives outward migration of the spindle during *C. elegans* meiosis

Alma Aquino, Francis J McNally University of California, Davis - Davis, CA

Cortical positioning of the meiotic spindle within an oocyte is required to expel chromosomes into polar bodies to generate a zygote with the correct number of chromosomes. In *C. elegans*, yolk granules and mitochondria are packed inward, away from the cortex while the spindle moves outward, both in a kinesin-dependent manner. The kinesin-dependent inward packing of yolk granules suggests the existence of microtubules with minus ends at the cortex and plus ends extending inward, making it unclear how kinesin moves the spindle outward. We hypothesize that inward packing of organelles might indirectly force the spindle outward by volume exclusion. To test this hypothesis, we generate a strain in which the only kinesin consists of motor domains with no cargo-binding tail optogenetically attached to mitochondria. This mitochondria-only kinesin packs mitochondria into a tight ball and efficiently moves the meiotic spindle to the cortex, supporting the volume exclusion hypothesis.

186A Temporal Regulation of Longevity Due to Loss of OXPHOS Subunits

Seth M Ashby[1], Melisa Matonsi[2], Kirin Guay[2], Timber Mattson[2], Suzanne Angeli[21]Molecular & Biomedical Sciences, University of Maine, [2]University of Maine

Mitochondrial activity remains a determining factor in the aging process as well as the onset of several age-related diseases, such as Alzheimer's and Parkinson's disease. We recently identified that the temporal loss of the F-ATP synthase subunit OSCP/atp-3 has varying effects on longevity in C. elegans. Specifically, we found that lifelong loss of OSCP/atp-3 (including during development) extends lifespan while during adulthood shortens lifespan. Previous studies have shown that loss of OXPHOS subunits during development only is sufficient to leave long-lasting effects on longevity (Durieux et al., 2011). In this study, we tested the temporal effects of the loss of other subunits from F-ATP synthase. RNA interference (RNAi) was used to block the transcriptional expression of F-ATP synthase genes, and survival analysis was conducted to determine how the lifespan of C. elegans was affected. We blocked the expression of F-ATP synthase genes atp-1, atp-2, atp-3, R04F11.2, R53.4, Y82E9BR.3, and asb-2, which correspond to the human homologs ATP5F1A, ATP5F1B, ATP5PO, ATP5J, ATP5H, ATP5G1, and ATP5I, respectively. We found that lifelong loss of the genes of interest increased the lifespan by about 53% (atp-1), 54% (atp-2), and 50% (atp-3), compared to controls. We are currently testing the effects of loss of F-ATP synthase subunits during additional developmental time periods only. Results from this study will help determine if developmental rewiring due to loss of OXPHOS subunits generally leads to longevity.

187A Expression patterns of EGFR ligands in *C. elegans*

Marine Barsegyan[1], Cheryl Van Buskirk[21]Biology, California State University, Northridge (CSUN), [2]Department of Biology, California State University, Northridge (CSUN)

Epidermal growth factor (EGF) family ligands are key mediators of intercellular communication across species. C. elegans LIN-3 is a well-characterized EGFR ligand that plays a role in specific developmental events, and this is reflected by its restricted expression in a subset of cells, such as the anchor cell (AC), pharynx, spermatheca valve, and others (Hwang and Sternberg 2004).

However, LIN-3 is not the only functional LET-23/EGFR ligand. SISS-1 is a more recently characterized EGF ligand that plays a central role in stress-induced sleep (SIS). As SIS can be triggered quickly by damage to a variety of tissues, we predict SISS-1 to be constitutively and widely expressed. Our transgenic and endogenous siss-1 transcriptional reporters partially confirm this prediction. We observe a continuous SISS-1 expression in a wide range of tissues except for body wall muscle. Interestingly, we find unexpectedly high levels of SISS-1 expression in rather specific cells and tissues, such as the pharynx, the rectal gland, a subset of primary vulval cells (vulF), the distal tip cell, and vulval muscles (vm1). It remains unclear whether these tissues are more competent than others in contributing to stress-induced sleep or whether SISS-1 serves an alternative function in these cell types.

IGEG-2 is another EGF family ligand, and our lab found that it is capable of activating LET-23/EGFR when overexpressed. Loss-of-function of IGEG-2 does not result in obvious developmental or behavioral defects. To shed light on its endogenous function, we constructed an igeg-2::gfp transcriptional reporter and observed that IGEG-2 is rather specifically localized to the excretory canal cell—a renal-like structure that plays a role in osmoregulation. Despite this distinct expression, we have not detected significant differences in canal morphology or function. We are currently investigating the potential function of this EGFR ligand.

188A The microfluidic *C. elegans* imaging toolbox

Simon Berger[1], Silvan Spiri[1], Andrew deMello[2], Alex Hajnal[11]University of Zurich, [2]ETH Zurich

Traditionally, *C. elegans* is immobilized during imaging using agar pads. However, agar-pad immobilization negatively impacts development, and preparing and using agar pads is labor-intensive and time-consuming. Over the past years, we have developed a comprehensive toolkit to address these limitations through various microfluidic systems for long-term and automated high-throughput imaging.

First, we developed long-term imaging strategies using devices allowing imaging of multiple animals across all larval stages (Berger *et al.* 2021). Animals are continually housed in an array of parallel trap channels, designed such that animals can grow and molt unimpeded. Multiple animals can be imaged in a single field of view and animals are fully immobilized during image acquisition, allowing for reliable, high-resolution imaging and tracking of complex developmental processes that were previously inaccessible.

Second, we developed an efficient, easy-to-use imaging method utilizing a similar parallel trap channel array (Spiri *et al.* 2022). High throughput is achieved by imaging multiple animals within a single field of view, either manually on any commercial microscope or using a custom microscope system capable of automatically imaging several thousand animals per hour. This method dramatically accelerates routine imaging speed and permits large-scale imaging screens, facilitating scientific discovery at a faster pace and resolution than previously possible.

Finally, we illustrate the utility of both platforms in various applications. Long-term imaging throughout post-embryonic development enables the observation of dynamic processes such as asymmetric seam cell divisions or vulval morphogenesis. Automated high-throughput imaging of over 300,000 animals identified several mutations by forward genetics and small molecules in compound screens, which we demonstrate in screen identifying genes inhibiting anchor cell invasion in a large scale RNAi screen, and small molecule compounds inhibting RAS/MAPK signalling in a large scale drug screen.

189A LGL-1 and the RhoGAP protein PAC-1 act redundantly to control apical-basal polarity in the embryonic epidermis

Olga Jarosinska[1], Amalia Riga[1], Hala Fahs[2], Ruben Schmidt[1], Fathima S Refai[2], Suma Gopinadhan[2], Kristin C Gunsalus[2,3], Mike Boxem[11]Biology, Utrecht University, [2]Center for Genomics and Systems Biology, New York University Abu Dhabi, [3]3.Department of Biology and Center for Genomics and Systems Biology, New York University

The formation of an apical–basal axis of polarity is essential for the organization and functioning of epithelial cells. Apical–basal polarity is established by cortical polarity proteins that define the apical, junctional, and basolateral domains. While cortical polarity proteins are highly conserved and present in most epithelia, the mechanisms that establish polarity and the requirements for canonical polarity factors can vary between tissues and organisms. For example, the basolateral polarity protein lethal giant larvae (Lgl) and the apical determinant Crumbs are essential for the polarization of most *Drosophila* epithelia, yet are dispensable for epithelial polarization and viability in *C. elegans*.

To better understand the epithelial polarity program in *C. elegans*, we performed a whole-genome RNAi screen for synthetic lethality with an *lgl-1* deletion mutant. We found that combined loss of LGL-1 and the RhoGAP protein PAC-1 leads to embryonic lethality due to defects in elongation and rupturing of the epidermis. We observed mislocalization of the junctional proteins DLG-1, HMR-1, and AFD-1 to the lateral domain of epidermal cells, presumably weakening tissue integrity. Furthermore, we observed expansion of the apical domain, sporadic PKC-3 mislocalization, and localization of the basolateral polarity protein LET-413 in patches surrounded by DLG-1. These observations indicate that the combined loss of LGL-1 and PAC-1 leads to an overactivity of apical domain specifying factors.

We next investigated the effect on *lgl-1(null); pac-1(RNAi)* animals of reducing the activities of three apical polarity regulators: the presumed LGL-1 target PKC-3; the PKC-3 activator and PAC-1 target CDC-42; and the putative PKC-3 activator and Crumbs family member CRB-3. The reduction of activity of each of these three proteins reduced the lethality of *lgl-1(null); pac-1(RNAi)* animals. Taken together, our data show that *pac-1* and *lgl-1* redundantly act to inhibit apical polarity specification factors. Furthermore, they show that, though their relative importance within the epithelial polarity program differs from that in fly epithelia, the canonical apical–basal polarity machinery is active in the embryonic epidermis of *C. elegans*.

190A *C. elegans* huntingtin, *htt-1*, promotes robust autophagy induction and survival under stress conditions

Christine H Chung[1,2], Hanee Lee[2], Kyumin Park[2], Young Seo Park[3], Roy Jung[4,5], Ihnsik Seong[4,5], Junho Lee[1,2,61]Department of Molecular Biology and Genetics, Seoul National University, [2]Institute of Molecular Biology and Genetics, Seoul National University, [3]Department of Molecular Biology and Genetics, Cornell University, [4]Center of Human Genetic Research, Massachusetts General Hospital, [5]Department of Neurology, Harvard Medical School, [6]Department of Biological Sciences, Seoul National University

Huntingtin (HTT) is the gene responsible for Huntington's disease (HD), a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion mutation. While HD pathogenesis has traditionally been attributed to the toxic gain-of-function effects of mutant huntingtin (mHTT), increasing evidence underscores the critical role of wild-type HTT loss-of-function. Understanding the physiological roles of HTT is essential for elucidating HD mechanisms and developing effective therapeutic strategies. The C. elegans *htt-1* gene, an ortholog of human HTT, remains largely uncharacterized. Here, we demonstrate that *htt-1* promotes survival under stress conditions that requires autophagy as a defense mechanism. Specifically, we identify intestinal *htt-1* as a key regulator of *C. elegans* survival during *Pseudomonas aeruginosa* PA14 infection. Our findings reveal that *htt-1* functions downstream of the MPK-1/ERK pathway to induce systemic autophagy and enhance host defense during immune challenges. Moreover, expression of wild-type human HTT in *htt-1* mutant worms rescues the survival defect during PA14 infection. Expression of mutant human HTT, on the other hand, exacerbates survival deficits, underscoring the conserved function of HTT across species. Additionally, we found that *htt-1* affects survival and autophagy under heat shock stress conditions. These results establish *htt-1* as a critical regulator of survival and autophagy in response to both pathogenic bacterial infection and thermal stress.

191A Non-autonomous TIR-1/SARM1 and PMK-1/p38 MAPK orchestrate homeostatic downregulation of germline stem cell proliferation in *C. elegans*

Alexandre Clouet, Matthieu Valet, Benjamin Dufour, Janina Rieger, Armi M Chaudhari, Lloyd Venceslas Fotso Dzuna, Xavier Lechasseur, Alexane Murray, Patrick Narbonne Département de Biologie Médicale, Université du Québec à Trois-Rivières

Stem cells generate differentiated cell types required for organ function and homeostasis. Precise regulation of stem cell proliferation must be maintained to prevent pathologies like cancers. In adult hermaphrodite *C. elegans*, the rate of germline stem cell (GSC) proliferation is minimally regulated by two factors: nutrient uptake, and the quantity of mature oocytes. When sperm is present, GSCs proliferate to provide new oocytes at a fast pace. When sperm is absent or oocytes are unfertilizable, oocytes accumulate and promote GSC quiescence, altogether preventing oocyte hyperaccumulation. This homeostatic feedback needs to integrate oocyte abundancy and signal across multiple tissues to modulate GSC proliferation. We know that homeostatic signaling must inhibit MPK-1, which acts in the gut or gonadal sheath cells to promote GSC proliferation. Through a forward genetic screen, we isolated two *tir-1* loss-of-function alleles that each resulted in oocyte hyperaccumulation in the *oma-1; oma-2* background. We targeted all known *tir-1* interactors using RNAi and/or alleles and identified the conserved NSY-1 -> SEK-1 -> PMK-1 stress-activated p38 MAPK module as being required for homeostatic signaling. In addition to previously identified intestinal, neuronal and hypodermal expression, we observed that endogenous PMK-1 is also present in the somatic gonad, including in the sheath cells and spermatheca. We thus asked where p38 MAPK activity was required to prevent oocyte hyperaccumulation in the absence of sperm. Classical transgenic *sek-1* rescue experiment with intestinal (*ges-1*), neuronal (*unc-119*) or sheath cells (*lim-7*) specific promoters, indicated that sheath *sek-1* was sufficient to rescue homeostatic downregulation of GSC proliferation in feminized *sek-1* doubles. We next asked whether *pmk-1*/p38 was acting upstream of *mpk-1*. Using epistasis analyses, we observed that *mpk-1; nsy-1* and *mpk-1; sek-1* doubles displayed low GSC proliferation rates like *mpk-1* singles, suggesting that p38 MAPK functions upstream MPK-1 in homeostatic signaling. Altogether, our results suggest that oocyte accumulation triggers a PMK-1-mediated stress response in sheath cells that leads to homeostatic inhibition of MPK-1 to downregulate GSC proliferation; and highlight a novel role for TIR-1 and p38 MAPK. If conserved, this phenomenon could underlie homeostatic regulation of stem cells in other species, and its disruption could lead to stem cell overproliferation and tumorigenesis.

192A Characterizing the sex-specific role of SYP-6 in spermatocyte heat sensi=vi

Karissa S Coleman[1], Diana E. Libuda[2], Cori K. Cahoon[31]Biochemistry and Molecular Biology, Colorado State University, [2]University of Oregon, [3]Colorado State University

Environmental stressors such as changes in chemical exposure, pH, and temperature can impact genomic stability of developing gametes. Unlike oocytes, spermatocytes are particularly sensitive to acute heat exposure, which can impair fertility through accumulation of DNA damage. In *Caenorhabditis elegans,* sex-specific regulation of the highly conserved synaptonemal complex (SC) likely contributes to the heat sensitivity of spermatocytes. The SC assembles between homologous chromosomes at the start of meiosis and functions to regulate meiotic events critical for both egg and sperm fertility. When exposed to heat-stress, we find that only spermatocytes display elevated amounts of DNA damage and SC stability defects that impact both SC assembly and disassembly. Notably, the composition of the SC is sexually dimorphic. Specifically, the temporal regulation of the SC protein SYP-6 is different with SYP-6 disassembling from the SC earlier in oocytes than spermatocytes. Our preliminary data indicates that heat stress only alters spermatocyte SYP-6 localization with SYP-6 being removed from the SC prematurely following an oocyte-like timing of disassembly. Here, we aim to elucidate the sex-specific impact SYP-6 has on heat induced DNA damage and the temperature sensitivity of the SC. We used immunofluorescent imaging to visualize both the SC and the amount of DNA damage in a *syp-6* mutant with and without heat-stress. We found that loss of SYP-6 has little impact on the SC temperature sensitivity as the heat-dependent loss of the SC is no different from wildtype. However, *syp-6* mutants displayed elevated amounts of DNA damage at non-heat conditions in only spermatocytes suggesting a sex-specific role for SYP-6 in meiotic DNA repair. Additionally, we also found that SYP-6 suppresses heat induced DNA damage in oocytes, but not spermatocytes. Taken together, these results suggest that SYP-6 may have dual sex-specific roles in both regulating DNA repair and the preventing heat induced DNA damage.

193A Repairing a broken gut: PAR-3 re-expression enables L1 larvae with cystic guts to repair into a functional and continuous gut

Lauren Cote[1], Melissa Pickett[2], Jessica Feldman[11]Stanford University, [2]College of Idaho

Continuity of the apical surface of a lumen is a crucial feature of epithelial cells. Diseases such as congenital atresias and polycystic kidney disease occur when epithelial cells form closed off cysts instead of continuous lumens. Initially, we erroneously assumed that development of cystic structures would result in permanent defect, however we will describe a case of a congenital cystic defect that is able to repair into a functional structure. In PAR-3gut(-) embryos, gut cells fail to form continuous apical surfaces and 100% of the resulting hatched die as arrested L1 larvae. This defect is specific to the time window of polarization in the embryo, as later depletion of PAR-3 does not compromise gut function. However, we found that re-expression of PAR-3 (PAR-3embryo gut(-)) after polarization surprisingly results in repair of cystic guts and many larvae grow into viable, fertile adults over ~7 days. Degradation under a gut-specific promoter (background expression from a tetO promoter; 'embryo gut(-)') that specifically turns on in a pulse of expression during embryogenesis enables the establishment of genetically facile lines that reproducibly yield repaired larvae. To confirm that the repair is PAR-3-dependent, exogenous expression of PAR-3 after polarization in our strongest PAR-3gut(-)background also resulted in larval gut repair. Finally, this repair process occurs in the absence of cell division and is likely to involve cell-cell junction exchanges to resolve the cysts. Ongoing visualization of this process will allow us to ask questions such as whether epithelial repair can be modeled as fluid flow, how cell-cell junction exchanges are coordinated by PAR-3, and what physical properties are associated with larvae that repair versus larvae that remain cystic. Our preliminary data indicate that junctional proteins may be inhibitory to continuous lumen formation in this context, indicating that there is careful interplay between apical and junctional surfaces to form a continuous lumen between neighboring cells. By leveraging prior and ongoing work on embryonic apical polarization and this new congenital repair context, we aim to compare the cellular and molecular mechanisms that ensure the formation of a continuous apical surface across multiple cells to better understand how spatial information is used to properly localize apical surfaces and ensure luminal continuity.

194A Alternative meiotic DNA repair pathways coordinate the repair of heat-induced DNA double strand breaks in *C. elegans* spermatogenesis

Caroline A. M. Crahan, Diana E. Libuda University of Oregon

Precise coordination of specific DNA damage repair pathways within developing sperm and eggs is critical to preserve genomic integrity through generations. In many organisms, spermatogenesis is particularly sensitive to heat exposure relative to oogenesis, generating excessive DNA damage only in developing sperm and reduced male fertility. Previous work in *Caenorhabditis elegans* found that acute heat shock causes up to a 40-fold increase in DNA double strand breaks (DSBs) in spermatocytes, yet male infertility only increases 6-fold, suggesting the majority of the DSBs are effectively repaired. Further, the meiotic stage when the majority of heat-induced DSBs arise as well as the speed at which heat-induced DSB repair progresses indicate that homologous recombination is not the major repair pathway utilized for heat-induced DSB repair. Here we show that upon acute heat stress, multiple alternative meiotic DNA repair pathways become engaged to repair heat-induced DSBs specifically in spermatocytes. Using a combination of genetics and immunofluorescence in *C. elegans*, we find that sister chromatid repair (intersister repair) and error-prone repair pathways that are typically repressed in meiosis (*i.e.* non-homologous end joining (NHEJ), theta-mediated end joining (TMEJ), and single strand annealing (SSA)) play extensive roles in the rapid repair of heat-induced DSBs in spermatocytes. Moreover, we find that BRCA1/BRC-1-mediated intersister repair is one of the major pathways coordinating heat-induced DSB repair and is responsible for protecting male fertility upon heat shock. Taken together, this study identifies how developing sperm cope with heat stress through the usage of alternative meiotic DNA repair pathways to adapt to extreme environmental changes to preserve genomic integrity during spermatogenesis.

195A A novel tethering mechanism that drives the autophagosome-phagosome fusion in *Caenorhabditis elegans*

Omar U Cruz Ramirez[1,2], Omar Pena-Ramos[2], Lucia Ciao[2], Frank Lu[2], Zheng Zhou[21]BioSciences, Rice University, [2]Baylor College of Medicine

Apoptosis and cell corpse removal play a crucial role in development and overall, in homeostasis. In *Caenorhabditis elegans*, apoptotic cells are recognized by neighboring cells via the CED-1 receptor, the engulfing cell membrane subsequently invaginates around the cell corpse and forms a single-membrane vesicle known as phagosome. Degradation of the phagosomal content relies on the fusion between a phagosome and lysosomes, which deliver hydrolases for the breakdown of the cargo. Autophagy is a different eukaryotic cleanup procedure, in which the endogenous waste is collected into the double-membrane vesicles known as autophagosomes. Subsequently, autophagosomes fuse to lysosomes, resulting in the degradation of the autophagosomal content. Despite the similarities, little overlap in the progression of phagocytosis and autophagy was described. We previously reported the novel discovery of the fusion between autophagosomes and phagosomes (AP fusion), an event that contributes to phagosome maturation and facilitates the clearance of apoptotic cells in *C. elegans*. However, the molecular mechanisms driving this unique interaction remain elusive. According to the well-recognized model in the literature, prior to the fusion of two intracellular organelles, multi-subunit tethering complexes mediate the initial fastening of two membranes by binding to Rab GTPases and effector proteins on the surface of the interacting vesicles. The HOPS/RAB-7 axis is probably the most studied tethering system, as it drives the fusion of lysosomes with both phagosomes and autophagosomes. However, in stark contrast to their well-known tethering roles, we showed that autophagosomes attach to phagosomes and accumulate on their surface in the absence of HOPS/RAB-7. In this sense, we propose a RAB-7-independent tethering mechanism to explain the progression of the AP fusion. This model stars RAB-2, RAB-14 GTPases, and probably their effectors. Using genetic approaches and time-lapse imaging, here we reveal novel tethering roles for candidates, as well as their involvement in phagosome maturation and corpse clearance, where they promote the recruitment and retention of RAB-7 on the phagosome surface. Moreover, RAB-2 and RAB-14 seem to work in parallel to recruit RAB-7. We are investigating how and why two members of the RAB family of small GTPases act together to promote autophagosome attachment on phagosomes and the recruitment of RAB-7.

196A Loss of a VIMP ortholog in *C. elegans* reduces clearance of misfolded proteins at the endoplasmic reticulum (ER) but does not induce expression of an ER stress reporter

Karla Acevedo[1], Callie Seymour[1], Anna Byquist[1], Matthew Zinkgraf[1], Lars Ellgaard[2], Caroline L Dahlberg[1]1Biology, Western Washington University, [2]Biology, University of Copenhagen

The Valosin-containing Protein-interacting Membrane Protein (VIMP, SelenoS) is a member of the ER-Associated Degradation (ERAD) machinery that can interact directly with the adaptor protein, p97, which shuttles misfolded proteins to the proteasome for degradation. Despite VIMP's central position in ERAD protein complexes, its precise roles in ERAD and cellular stress responses are still unclear. VIMP levels increase during ER stress, and some studies suggest that cells that lack VIMP have reduced response to ER stress, as measured by the induction of Unfolded Protein Response (UPR) genes including xbp1 and hspa5 (BiP). In *C. elegans*, a putative ortholog to VIMP is the gene F26F4.9. F26F4.9 has high sequence homology with mammalian VIMP except for at its C-terminus, where it contains a glutaredoxin domain instead of the unstructured selenocysteine-containing domain. We find that genetic deletions in F26F4.9 (*tm2430* and *tm2433*) leads to accumulation of the ERAD substrate, CPL-1W32A;Y35A::YFP, suggesting that the VIMP ortholog is required for normal ERAD function. However, the ER stress reporter transgene, P*hsp-4*::GFP, is not activated in response to the loss of F26F4.9. Additionally, we find that the *tm2430* allele of F26F4.9 reduces the activation of P*hsp-4*::GFP that usually occurs when animals are treated with the ER stress inducing drug, Tunicamycin. Futhermore, while RNAi against *hsp-4* dramatically increases the activity of GFP expressed under the *hsp-4* promoter, loss of F26F4.9 reduces P*hsp-4*::GFP expression by 50%. Using transcriptomic analysis, we have identified two classes of genes whose regulation in response to Tunicamycin is modified by the absence of F26F4.9. Our current work addresses the genetic mechanisms by which F26F4.9 may work within the ERAD machinery and through transcription as part of the unfolded protein response.

197A The acyl-CoA dehydrogenase ACDH-11 functions in the formation of lysosome-related organelles

Madeline Daniel, Trent Nause, Bella Root, Emogene Lutz, Frances Parrott, David Nhek, Greg Hermann Lewis & Clark College

Gut granules are lysosome-related organelles that coexist with conventional lysosomes within *C. elegans* intestinal cells. Gut granules contain crystalline and autofluorescent material and function in lipid metabolism, cell signaling, and heme and micronutrient storage. The formation of gut granules requires evolutionarily conserved proteins that broadly function in LRO biogenesis. From screening Million Mutation Project strains we identified a role for the ACDH-11 acyl-CoA dehydrogenase in gut granule formation and morphology. *acdh-11(-)* mutant embryos exhibit enlarged gut granules, defects in the steady-state localization of gut granule associated proteins, and the loss of ArfGAP localization to the Golgi. The morphology of conventional degradative lysosomes, lipid droplets, and yolk granules are unaltered in *acdh-11(-)* mutants. ACDH-11 activity has been shown to inhibit the expression of the lipid desaturase FAT-7 in adult intestinal cells. We find that *acdh-11* is expressed in embryos and that FAT-7 becomes expressed in embryonic intestinal cells in *acdh-11(-)* mutants. The removal of *fat-7* activity in *acdh-11(-)* mutants restores proper gut granule size and gut granule protein trafficking. The overexpression of FAT-7 in *acdh-11(-)* results in gut granule enlargement, showing that FAT-7 activity promotes the defects in gut granule formation in *acdh-11(-)*. FAT-7 catalyzes the generation of unsaturated fatty acids to increase membrane fluidity. Consistent with increased membrane fluidity promoting gut granule enlargement, gut granules in *acdh-11(-)* mutants are significantly smaller at 15C compared to 22C. Recent work suggests that ACDH-11 functions in the metabolism of cyclopropyl fatty acids (Fox et al. Nat Commun. 15:1520), which are abundant in OP50. Consistent with ACDH-11 participating in the beta oxidation of these lipids, we find that ACDH-11::GFP is localized to mitochondria. Additionally, we show that gut granule biogenesis defects in *acdh-11(-)* results from a buildup of these the fatty acids. Gut granule loss mutants are synthetically lethal with *acdh-11(-)* mutants when animals are fed cyclopropyl fatty acids, consistent with gut granules functioning in their storage or processing. We will present work that investigates how ACDH-11 impacts gut granule biogenesis and activity.

198A PLAA/UFD-3 regulates P-bodies through its intrinsic disordered domain

Alakananda Das[1], Yanping Qiu[2], Trevor J Wolf[2], Ella Brissett[2], Jae Hyoung Cho[2], Heenam Park[2], Eugene C Chen[2], Tsui-Fen Chou[2], Paul W Sternberg[2]1Biology and Biological Engineering, California Institute of Technology, [2]California Institute of Technology

Regulation of proteome homeostasis is crucial for the survival and adaptation to changing environments for all species. In eukaryotes, this process is finely tuned through regulation at the level of transcription, translation, protein modification and protein degradation. The Phospholipase A2 Activating Protein (PLAA) is present in all eukaryotes and believed to be a key player in ubiquitin-dependent protein sorting and degradation via its interactions with ubiquitin and/or the AAA+ ATPase, Valosin containing protein (VCP/p97). PLAA's molecular targets and interaction network remain unclear. We used *Caenorhabditis elegans* and unbiased proteome-scale approaches to investigate neuronal specific interactors of the *C. elegans* PLAA ortholog UFD-3 (ubiquitin fusion degradation 3), its effect on ubiquitinated proteins, and global protein expression changes in an *ufd-3* mutant. We discovered that PLAA may play a unique role in P-bodies. Using biochemical analysis *in vitro* and fluorescence imaging in *C. elegans*, we show that UFD-3 directly interacts with the mRNA decapping complex regulatory subunit DCAP-1. UFD-3's intrinsic disordered region (IDR), which contains conserved amino acid motifs, is important for the recruitment of DCAP-1 to P-bodies. Lastly, we show that loss of the IDR does not affect UFD-3's role in sorting ubiquitinated proteins through the multivesicular body pathway. Collectively, our results suggest that UFD-3's role in P-bodies is distinct from its role in the ubiquitin-dependent protein degradation pathway and the IDR region is only critical for UFD-3 regulated P-bodies pathways. Thus, PLAA/UFD-3 might regulate the proteome via two distinct pathways: ubiquitinated protein turnover, as well as mRNA regulation through P-bodies.

199A The role of kinesin-1 in organizing the ER envelope around the meiotic spindle

Rebecca M Do[1], Alma Aquino[2], Francis McNally[11]Molecular and Cellular Biology, University of California Davis, [2]molecular and Cellular Biology, University of California Davis

The endoplasmic reticulum (ER) is organized by kinesin-1 in mammalian cells. In maturing C. elegans oocytes, the nuclear envelope, a specialized region of ER, fenestrates during ovulation of the oocyte into the spermatheca to allow entry of tubulin needed for meiotic spindle assembly. After the zygote ovulates into the uterus, the ER envelopes the meiotic spindle with a sheetlike morphology during metaphase and a tubular morphology during anaphase. During metaphase I, there is also a dramatic aggregation of ER at the two poles of the meiotic spindle. The purposes of the ER spindle envelope and the ER aggregation at spindle poles are poorly understood. To test the role of kinesin-1 in organizing metaphase spindle-associated ER, we first generated a germline null allele of unc-116 which encodes the kinesin-1 heavy chain by complementing the unc-116(gk5722) lethal deletion with an integrated unc-116::GFP array, duls1, that is silenced in the germline. We used time-lapse fluorescence microscopy to track spindle microtubules labeled with mKate::tubulin and the ER transmembrane protein, TMCO-1::GFP in the kinesin null (n=5) and wild type (n=4) backgrounds. In the kinesin null background, the ER failed to form aggregates concentrated at the poles of the metaphase I spindle and instead formed aggregates that surrounded all sides of the spindle. We are currently increasing the number of time-lapse sequences to verify the requirement for kinesin-1 in concentrating ER aggregates at spindle poles. To test whether concentration of ER aggregates at spindle poles is important for meiotic spindle structure, we are also in the process of measuring metaphase spindle lengths. To ensure our results are not caused by the TMCO-1 fusion protein, we are also doing identical analysis with a second ER membrane protein, TMEM-147::GFP.

200A Exploring the Role of the Kinesin-8 KLP-13 and its Relationship to the NIMA-related Kinase NEKL-4 in Maintaining Ciliary Structure

Leah M Dobossy[1], Kaiden M Power[2], Maureen M Barr[11]Genetics, Rutgers University, [2]Genetics, Boston Children's Hospital

Cilia are microtubule-based organelles present in most cell types. Defects in cilia formation or function contribute to syndromic diseases termed ciliopathies, which have a range of symptoms including neurodegeneration, which may be caused by defects in microtubule-based processes. Microtubules incur tubulin post-translational modifications (PTMs) that regulate many functional aspects of ciliary microtubules. Tubulin PTMs and the enzymes that write, read, and erase PTMs are collectively called the Tubulin Code. One such tubulin PTM that is enriched in cilia is polyglutamylation, which is deposited by TTL (tubulin tyrosine ligase) enzymes and removed by CCP (cytosolic carboxypeptidase) enzymes. In humans, microtubule hyperglutamylation caused by mutations in CCP1 cause infantile-onset neurodegeneration. In *C. elegans,* mutations in the CCP1 homolog *ccpp-1* cause defects of the B-tubules in ciliary microtubule doublets region and progressive ciliary degeneration (measured by the dye filling defective Dyf phenotype). *ccpp-1* ciliary defects are partially suppressed by loss of the NIMA-related kinase, NEKL-4. Activation of NEKL-4 by overexpression or deletion of the PEST domain causes a similar progressive ciliary degeneration phenotype and disrupts microtubule ultrastructure, suggesting that NEKL-4 activity is important for cilia stability. The *nekl-4(PESTΔ)* mutant phenotype is partially suppressed by a predicted loss-of-function mutation in the kinesin-8- KLP-13. Kinesin-8 motors are unique in that they possess both plus-end directed motor and microtubule depolymerizing activity. In yeast and mice, kinesin-8 motors play roles in mitotic spindle organization. Mammalian kinesin-8 KIF19A localizes to ciliary tips and negatively regulates cilia length, likely due to its depolymerizing function. Park et al., 2021 showed that in *C. elegans,* ectopically-expressed KLP-13 localizes to phasmid ciliary tips and negatively regulates ADL cilia length. Here, we show using an endogenous CRISPR knock-in tagRFP reporter that KLP-13 localizes to ciliary tips in the nose of the worm. We are currently determining what cell types express *klp-13*. In addition, we aim to test the hypothesis that NEKL-4 and KLP-13 work in concert to regulate tubulin polyglutamylation in the labial and amphid cilia. Our goal is to determine the role of KLP-13 and its relationship to NEKL-4 in maintaining ciliary structure, possibly through modulation of the Tubulin Code.

201A Investigating the role of the AFD cilium in regulating AFD sensory ending structure and function

Priya Dutta, Piali Sengupta Department of Biology, Brandeis University

The AFD thermosensory neuron pair in *C. elegans* responds to environmental temperature changes in an experience-dependent manner to guide thermotaxis navigation behavior. The AFD sensory ending consists of two sensory compartments, a short microtubule-based cilium and hundreds of actin-based microvilli, finger-like projections that surround the cilium. Thermotransduction molecules are differentially distributed in these sensory compartments. For example, the thermoreceptor guanylyl cyclases (GCY-8, GCY-18 and GCY-23) and the SRTX-1 GPCR localize to the AFD microvilli, whereas the thermotransduction channel TAX-4 localizes to the base of the AFD cilium (Nguyen et al., 2014). The functional significance of two distinct sensory compartments and the distinct subcellular localization patterns for thermotransduction molecules for AFD thermosensory responses is unclear. To address this issue, we are specifically disrupting the AFD cilium and investigating its effects on microvilli structure and AFD function. Loss-of-function mutations in the *daf-19* RFX transcription factor result in the absence of sensory cilia (Swoboda et al., 2000). In *daf-19* mutants, we find that the morphology of AFD microvilli is extensively altered. Loss of the AFD cilia also leads to changes in the temperature-evoked experience-dependent calcium responses in AFD. Previous studies have shown that the microvilli form after the AFD cilium and could be a ciliary subcompartment (Nguyen et al., 2014). Therefore, we are also examining the mechanisms by which the development of cilia and microvilli are coordinated to form the unique AFD sensory ending structure. Our results suggest that the AFD cilia may maintain the microvilli structure, possibly by fine tuning the thermosensory signaling cascade.

Reference:

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Nguyen, P. A. T., Liou. W., Hall, D. H., Leroux, M.R. (2014). Ciliopathy proteins establish a bipartite signaling compartment in a *C. elegans* thermosensory neuron. *J Cell Sci* 15, 5317–5330.

202A The nature of mitochondrial DNA deletions dictates the cellular response to heteroplasmy

Samantha Fiallo, Jocelyn Sanchez, Will Carlen-Jones, Sagen Flowers, Cricket Wood, Pradeep Joshi, Joel Rothman UC Santa Barbara

The heteroplasmic state of mitochondria allows defective mitochondrial DNA (mtDNAs) to accumulate in eukaryotic cells. mtDNA is especially susceptible to damage by reactive oxygen species that are a byproduct of oxidative phosphorylation. This problem is particularly severe when damage leads to mtDNA deletions, as such mutants can gain replicative advantage over intact mtDNA. Such deleted mtDNAs can also lead to imbalances in mitochondrial protein homeostasis. The mitochondrial unfolded protein response (UPRmt) in *C. elegans* has been shown to be triggered by, and required for, maintenance of a 3.1 kb mtDNA deletion, *uaDf5* in stable heteroplasmy with intact mtDNA. In the absence of UPRmt function, this mtDNA deletion is lost. The *uaDf5* deletion causes an in-frame fusion protein formed between truncated ND1 and Cyt b coding regions and also removes 7 tRNAs. Either one or both of these effects might be responsible for triggering the UPRmt. We sought to examine whether UPRmt is induced as a result of general loss of mitochondrial function or instead occurs in response to loss of specific mtDNA sequences. To do so, we monitored induction of an HSP-6::GFP reporter of UPRmt activity in six mtDNA deletions obtained from the Million Mutation Project that remove different regions of mtDNA. All of the deletions that remove tRNAs, including one that eliminates six tRNAs but does not result in a truncated or fusion protein, cause activation of the UPRmt reporter. In contrast, we found that two deletions that do not remove any tRNAs, including one that generates a truncated protein, did not activate the UPRmt reporter. These results suggest that imbalance in mitochondrial tRNA levels may be responsible for UPRmt induction and that the cell differentially responds to specific types of mtDNA damage to maintain heteroplasmy of different deletions, including through UPRmt-independent mechanisms. Indeed, fission and fusion dynamics of mitochondria have been shown to alter heteroplasmy levels, with fusion-defective *fzo-1* mutations leading to complete destabilization of *uaDf5*. Consistent with these observations, we found that the presence of *uaDf5* affects mitochondrial morphology by significantly decreasing the number of branches per mitochondria . Further, we discovered a spontaneous mutation, *w76*, that does not alter either *atfs-1*, which mediates the UPRmt, or *fzo-1*, and that completely destabilizes *uaDf5* and three other mtDNA deletions specifically. Our preliminary results indicate that this mutation eliminates these mtDNA deletions at different rates. These results provide evidence that the effect of an mtDNA deletion on protein balance within mitochondria mediates the specific program responsible for removing defective mtDNAs and maintenance of heteroplasmy levels.

203A Sexually dimorphic morphology and heat-sensitive localization of PRG-1 aggregates in the *C. elegans* germline

Lily J Francis, Nicole Kurhanewicz, Hannah R Wilson, Diana E Libuda Institution of Molecular Biology, University of Oregon

The maintenance of fertility in response to stress is critical for organism and population survival in the face of an ever-changing environment. Temperature fluctuation is omnipresent in the natural environment, and fertility is known to be sensitive to temperature stress. Across taxa, male fertility is particularly sensitive to elevated temperature, with acute heat exposure causing DNA damage specifically in developing sperm (not in oocytes or other tissues) and male infertility. The Caenorhabditis elegans PIWI/piRNA pathway, led by master regulator PRG-1, plays an important role in the maintenance of genomic integrity and regulation of gene expression in the germline. Further, the piRNA pathway is heat-sensitive, and has roles in the regulation of both sperm development and male fertility. As such, sexually dimorphic dysregulation of the PIWI/piRNA pathway represents a compelling mechanism underlying the production of heat induced DNA damage specifically in developing sperm. To assess sexual dimorphic aspects of the PIWI/piRNA pathway, we used high-resolution immunofluorescence microscopy to characterize PRG-1 protein aggregate localization and morphology during both C. elegans spermatogenesis and oogenesis. PRG-1 displays sexually dimorphic aggregate morphology and germline localization at baseline, as well as following heat shock. Notably, spermatocyte-associated PRG-1 aggregates are in greater number and more spherical than their oocyte-associated counterparts. In addition, ring-like PRG-1 aggregates are present in late pachytene at significantly higher incidence in spermatocytes vs oocytes. Following heat stress, PRG-1 aggregation is disrupted in both sexes, however spermatocyte-associated PRG-1 aggregates remain largely nuclei-adjacent, while heat disrupted oocyte-associated PRG-1 aggregates are largely cytoplasmic. Further, we found that knockdown of PRG-1 enhances the production of heat-induced DNA damage specifically in spermatocytes, indicating that the piRNA pathway is involved in the regulation of heat-induced DNA damage in sperm. Taken together, our ongoing work provides insight into sexual dimorphisms of germline genome maintenance programs, and the broader conserved mechanisms underlying the heat-induced male infertility.

204A Genetic Screen in *C. elegans* Uncovers Non-Canonical Autophagy Regulators

Joshua P Gill, Cristian Ricaurte-Perez, Olga Dubuisson, P. Kerr Wall, K. Adam Bohnert, Alyssa E Johnson Biological Sciences, Louisiana State University

Human lifespan has been on a steady increase over the past century, but human healthspan has not increased on the same trajectory, resulting in a larger population of humans that suffer from age-related diseases. Many age-related diseases result from increased protein aggregation or loss of proteostasis. Autophagy, which is one of the main protein degradation pathways, recycles cellular components via lysosomes; thus, lysosomes could be a major target for the treatment of age-related diseases. In recent work, we identified a class of non-canonical lysosomes that form tubular networks in the gut of starved or dietary restricted (DR) *C. elegans*. Significantly, induction of tubular lysosomes (TLs) increases autophagic potential and is a critical event in achieving the full beneficial effects of DR. In *Drosophila*, TLs can be stimulated ectopically by overexpressing the lysosomal gene *SVIP* (Small VCP Interacting protein). Although *C. elegans* do not have an *SVIP* ortholog, over-expression of either human or *Drosophila SVIP* in the gut of *C. elegans* resulted in many physiological improvements that mimic DR, including TL induction, increased autophagic cargo turnover, dramatic improvement of late-age mobility, and enhancement of late-age mitochondrial and muscle health. Thus, SVIP provides a route to stimulate TLs on demand and could be tapped for anti-aging interventions. To dissect the molecular mechanisms through which *SVIP* overexpression causes these effects, we performed mRNA sequencing from whole worms with and without *SVIP* overexpression, which resulted in 1375 upregulated genes compared to WT. Next, we employed an RNAi screening strategy to validate genes that disrupted autophagic cargo turnover in *SVIP* overexpression worms using the tandemly-tagged *SQST-1::mCherry::GFP* fluorescent marker. After screening 326 genes, we identified several genes whose inhibition resulted in defective autophagic cargo turnover, the most surprising of which were *ant-1.3/ant-1.4*, which encode ATP/ADP antiporter proteins that reside in the inner mitochondrial membrane and regulate ATP export and mitochondrial permeability. Using the loss-of-function mutant *ant-1.4(gk300)*, we validated that loss of *ant-1.4* caused a disruption of *SVIP*-dependent TL induction. The *ant-1.4(gk300)* mutant also reverted the thrashing rate of *SVIP* overexpression worms back to WT levels. We are now investigating how *ant-1.4* regulates lysosomes and autophagy and examining what effects *ant-1.4* has on the mitochondria. These studies could reveal a new connection between lysosomes and mitochondria.

205A rRNA intermediates coordinate the structure of nucleolus in *C. elegans*

Shouhong Guang University of Science and Technology of China

The nucleolus is the most prominent membraneless organelle within the nucleus. How the nucleolar structure is regulated is poorly understood. Here, we identified two types of nucleoli in *C. elegans*. Type I nucleoli are spherical and don't have visible nucleolar vacuoles (NoVs), and rRNA transcription and processing factors are evenly distributed throughout the nucleolus. Type II nucleoli contain vacuoles and rRNA transcription and processing factors exclusively accumulate in the periphery rim. The NoV contains nucleoplasmic proteins and are capable of exchanging contents with the nucleoplasm. The high-order structure of the nucleolus is dynamically regulated in *C. elegans*. Faithful rRNA processing is important to prohibit NoV. The depletion of 27SA2 rRNA processing factors resulted in NoVs formation. The inhibition of RNAP I transcription and depletion of two conserved nucleolar factors, nucleolin and fibrillarin prohibits the formation of NoVs. This found provide a mechanism to coordinate structure maintenance and gene expression.

206A Genomic and live imaging analysis of programmed DNA elimination in *Caenorhabditis auriculariae*

Nami Haruta[1], Naoki Uwatoko[1], Simo Sun[2], Taisei Kikuchi[2], Asako Sugimoto[1]1Graduate School of Life Sciences, Tohoku University, [2]Graduate School of Frontier Sciences, The University of Tokyo

Genomic information is generally identical across all cells of an individual. However, in certain organisms, specific chromosomal regions are selectively eliminated in the somatic cells during particular developmental stages—a phenomenon known as programmed DNA elimination (PDE). Although PDE was first observed 150 years ago in the horse parasitic nematode *Parascaris*, and recent advancements in whole-genome sequencing have revealed that some free-living nematodes also undergo PDE. However, the physiological significance and molecular mechanisms underlying PDE remain largely unknown.

Recently, long-read sequencing and Hi-C analysis revealed that three *Caenorhabditis* species undergo PDE (L. Stevens, et al., this meeting). Among them, we focused on PDE in *Caenorhabditis auriculariae* in this study.

The karyotype of *C. auriculariae* in germ cells is 2n = 12, as in most other *Caenorhabditis* species. However, in somatic cells, the karyotype is 2n = 26, as the terminal and internal regions of each chromosome, collectively accounting for 2% of the genome (2.4 Mb), are eliminated, while new telomeres are added to the breakpoints. The eliminated regions are rich in repetitive sequences and contain nearly 300 protein-coding genes.

We initiated elucidation of the molecular mechanisms underlying the PDE in *C. auriculariae*, using genetic techniques, including RNAi and transgenesis via microparticle bombardment. The live PDE process was observed for the first time using GFP::Histone. At the 8-cell stage, fragmented chromosomes were detected in somatic blastomeres before nuclear envelope breakdown (NEBD). Following NEBD, the DNA fragments destined for elimination localized to the outer edge of the metaphase plates and rapidly moved toward the cell cortex without being captured by spindle microtubules, leading to their exclusion from the nucleus. This exclusion process in *C. auriculariae* differs from PDE in some nematodes of other genera, such as *Ascaris*, in which the eliminated DNA remains in the midzone of segregated chromosomes during anaphase. This observation implies that the PDE in different nematode groups was acquired independently or that PDE processes can diverge rapidly. Currently, we are conducting RNAi knockdown screening and have identified several candidate genes involved in the PDE process. Thus, *C. auriculariae* serves as a powerful model system for understanding the mechanisms and evolution of PDE.

207A Investigating the initial formation and maintenance of gut granules

Madeline Daniel[1], Yoona Shim[2], Frances Parrott[2], Bella Root[2], Greg Hermann[2]1Biology, Lewis & Clark College, [2]Lewis & Clark College

Gut granules are intestinal lysosome-related organelles (LROs) that are distinct from but share some characteristics with conventional degradative lysosomes. Gut granules are homologous to mammalian melanosomes and their formation requires evolutionarily conserved proteins that broadly function in LRO biogenesis. Gut granules contain autofluorescent material and they function in metabolite storage, metabolism, and cell signaling. To determine how gut granules are first generated, we examined when organelles with gut granule associated proteins could first be detected during embryogenesis. We find no evidence that gut granules are present in oocytes or early-stage embryos. Instead, gut granules begin to be formed de novo when there are 4 descendants of the intestinal precursor E (the E4 stage), which represents one of the earliest aspects of intestinal cell differentiation. At the end of the E4 stage there are approximately 60 gut granules in the intestinal primordium. This increases to approximately 75 during at the E8 stage and then the number of gut granules remains at this level into the E16 stage throughout the rest of embryogenesis. We find that gut granules undergo a molecular and functional transition in the short period between the E8 and E16 stages that coincides with when their numbers plateau. In contrast to the E4 and E8 stages, gut granule numbers and size do not appreciably change through the latter half of embryonic development suggesting that gut granules are no longer being made or that there are similar rates of gut granule formation and removal. The Rab32/38 orthologue GLO-1 is required for the formation of gut granules. To examine how the steady state numbers of gut granules are maintained during later stages of embryogenesis we performed GLO-1 add back and GLO-1 removal experiments. This work involved developing a novel approach to specifically target the inactivation of GLO-1 using the Salmonella GtgE protease. We will present the results of these experiments and what they suggest about the mechanisms controlling gut granule maintenance. In addition, we will discuss evidence pointing toward a role for the GLO-1 Rab32/38 GTPase in retrograde trafficking out of gut granules.

208A Characterizing kinetochore component HCP-1 germline condensates and its role for successful cell division in *C. elegans*

Hannah L Hertz, Ian F Price, Wen Tang Department of Biological Chemistry and Pharmacology, The Ohio State University

The kinetochore is a protein complex that assembles on the centromere of chromosome. It functions as the attachment site for microtubules of the mitotic or meiotic spindle, promoting chromosome movement during cell division. Here we present evidence that kinetochore components, such as HCP-1, form distinct cytoplasmic foci in *C. elegans* germ line. Prior to cell division, HCP-1 localizes to the nuclear envelope of germ nuclei and forms phase-separated condensates that are distinct from germ granules. Upon mitotic entry, perinuclear HCP-1 is imported into the nucleus to facilitate chromosome segregation. We further show that HCP-1 condensates associate with nuclear pore proteins (NPPs) and that depletion of NPP-14 leads to the dispersal of perinuclear HCP-1. Our ongoing work aims to determine whether perinuclear HCP-1 condensates are required for its recruitment to kinetochores and the proper assembly of the central spindle. Together, these findings provide new insights into the spatial regulation of kinetochore components and their roles in nuclear organization and cell division.

209A Activity-dependent neuronal regulation of mitochondrial morphology and ROS production

Kaz M Knight[1], Zephyr Lenninger[1], Shan Su[2], Padmini Rangamani[2], Frederic J Hoerndli[1]1Biomedical Sciences, Colorado State University, [2]Department of Pharmacology, University of California San Diego School of Medicine

Neurons often have complex morphologies that promote and rely on compartmentalized signaling processes. Neuronal mitochondria play essential roles in compartmentalized signaling and localized energy production. Several studies in the last decade have shown an essential role for mitochondria in developmental and synaptic plasticity (1-3). However, how neuronal activity changes mitochondria morphology and function at different subcellular locations within excitatory neurons *in vivo* in mature adult neurons has not been systematically studied. Here using the glutamatergic command interneurons AVA, we investigate activity-dependent changes in somatic, proximal and distal mitochondria. We analyze differences in size, calcium uptake, reactive oxygen species production (mitoROS), as well as mobility and turnover. To manipulate AVA activity *in vivo*, we use either ASH presynaptic optogenetic stimulation with ChR2, direct AVA ChRimson stimulation, or olfactory conditioning. Our results indicate that 1 hour of physiological stimulation of pre(ASH)- or postsynaptic(AVA)lead to different mitochondrial adaptations in morphology and function. In addition, we show that the magnitude of these changes is location dependent and requires/relies on mitochondrial calcium uptake through MCU-1. To complement our experimental results, we turned to computational modeling using mitochondrial size, calcium influx and ROS production as inputs and stimulation or cytpoplasmic calcium and MCU-1 mitochondrial density as variables. We will present how modeling and in vivo experimental measures of mitochondrial morphology and function have informed us on the role of mitochondria signaling for neuronal activity and perhaps for synaptic plasticity. (1) Rangaraju et al 2023, PMID 30612742; (2) Virga et al 2024, PMID 38459070;(3) Kochan et al 2024, PMID 38582081.

210A Tetraspan protein SCAMP/SCM-1 maintains endosomal microdomains and membrane trafficking

Kam H Hu[1], Anne Norris[1], Barth D Grant[21]MBB, Rutgers, [2]Rutgers

SCM-1 is a tetraspan protein involved in regulating membrane trafficking in *Caenorhabditis elegans*. Characterized by four transmembrane domains, SCM-1 plays a crucial role in maintaining the integrity and organization of early endosomal microdomains. In the absence of SCM-1, a striking overlap occurs between the normally distinct degradative and recycling microdomains on early endosomes, disrupting the compartmentalization required for efficient trafficking. Despite this disruption, the levels of MIG-14, a cargo protein typically trafficked through these endosomal pathways, remain unchanged, though MIG-14-positive endosome numbers increase while their size decreases. This alteration is also observed with RAB-5 and LAMP-1-positive endosomes, which become smaller and more numerous in scm-1 mutants. In contrast, RAB-7-positive late endosomes are larger and less numerous in these mutants. Taken together, these findings suggest that SCM-1 may regulate the homotypic fusion of RAB-7 endosomes, preventing their fusion and promoting proper endosomal maturation or perhaps heterotypic fusion with the Lysosome. The observed phenotypes support the hypothesis that SCM-1's role in preventing Rab-7 homotypic fusion might be critical for favoring lysosome/Rab-7 fusion or maintaining other alternative endocytic trafficking pathways. These insights point to SCM-1 as a key regulator of endosomal dynamics in *C. elegans*, ensuring the efficient segregation and maturation of endosomal compartments.

211A Frequent paternal mitochondrial transmission in *Caenorhabditis briggsae* hybrids

Liliana Huerta Mora[1], Claire Irasusta[2], Julissa Topete Sanchez[1], Joseph Ross[11]Biology, California State University, Fresno, [2]California State University, Fresno

Most organisms inherit mitochondria only from their mothers. However, recent studies have revealed rare instances where paternal mitochondria are transmitted and persist in hybrid offspring of genetically diverse parents. Such paternal mitochondrial transmission (PMT) has been observed in organisms like mice, marine copepods, and *Drosophila*. We previously observed PMT in *Caenorhabditis briggsae* hybrid crosses, particularly in multi-generation serial backcrosses. This observation added to a list of studies that suggest PMT occurs most frequently in crosses between genetically diverse individuals. It is possible that a cellular mechanism preventing PMT becomes dysfunctional in hybrids and facilitates PMT. Our long-term goal is to identify the genetic loci and molecular mechanisms that regulate PMT. We first investigated whether paternal mitotypes can be identified in hybrids as early as the F1 generation: over 25% of F1 offspring had paternal mitochondria. We then hypothesized that alleles of unknown nuclear genes are incompatible in hybrids of genetically divergent *C. briggsae* strains. An incompatibility might cause hybrids of particular (but unknown) nuclear genotypes to facilitate PMT. To begin to map such loci, we crossed HK104 males with *C. briggsae* advanced intercross recombinant inbred line (AI-RIL) hermaphrodites, which have AF16 mitochondria and whose nuclear genomes comprise random blocks of HK104 and AF16 alleles. The F1 offspring were genotyped for the presence of the paternal HK104 mitotype. Screening offspring of multiple, genetically unique AI-RIL revealed that some AI-RIL genotypes facilitate PMT and others do not. In sum, PMT readily occurs in *C. briggsae* hybrids, it can be detected as early as the F1 generation, and it might have a complex genetic basis. Genetic mapping continues in the *C. briggsae* AI-RIL panel. This project provides insight into how uniparental mitochondrial inheritance is regulated, which could have an impact on data interpretation relying on the assumption of maternal mitochondrial inheritance in the analysis of phylogenies. Such knowledge might also be clinically relevant to help treat or prevent the transmission of mitochondrial genetic diseases.

212A The *C. elegans* WASH complex supports transport and microtubule function.

Patricia Irizarry[1], Bhagavathi Ramamurthy[2], Martha C Soto[31]Pathology, Rutgers University, [2]Department of Physician Assistant Studies Program, School of Health Professions, [3]Pathology, Robert Wood Johnson Medical School

C. elegans has three Nucleation Promoting Factors (NPSs) that regulate branched actin through Arp2/3, WAVE, WASP and WASH, but the WASH complex is minimally characterized. The WASH complex is similar to the WAVE complex, in that both complexes include 5 paralogous components. Surprisingly, only 4 of the 5 components of the WASH complex had been identified in C. elegans. We used existing mutations, RNAi and CRISPR to determine the effects of loss of the WASH complex on two epithelial tissues: the adult intestine and the embryonic epidermis. We used two phenotypic assays to test the role of WASH components, including a candidate for the missing 5th WASH component, the FAM21 proposed ortholog, CO5G5.2. (1) Assays for protein transport showed that loss of any WASH component, using mutations or RNAi, resulted in the same phenotype: defective transport of cargo on RAB-5 and PI(3)P-positive early endosomes. This result suggests C. elegans WASH regulates sorting during endocytic recycling, through retrograde trafficking to the Golgi apparatus. (2) Assays for embryonic development showed that loss of WASH components resulted in two types of embryonic arrest: during morphogenesis, and much earlier before morphogenesis begins. Therefore, in contrast to loss of WASP or WAVE, which are needed during morphogenesis, WASH is also needed to prevent cell cycle arrests. Beta-tubulin::GFP and alpha-tubulin::GFP expression was strongly diminished in embryos depleted of WASH components. It is known that the WASH complex assembles with the help of the microtubule (MT) motor dynein and its partner dynactin. Thus, MTs support WASH assembly. Our results suggest that WASH supports MT assembly. We are therefore investigating the role of WASH on MT function. Thus, we provide evidence that CO5G5.2 is the missing 5th member of the C. elegans WASH complex, and that C. elegans WASH functions, like its orthologs, in early endosome to Golgi retrograde transport. In addition, C. elegans provides a great system to investigate the coordination of WASH complex with MT-dependent events during early embryonic development.

213A Two tau tubulin kinases redundantly necessary for *C. elegans* sperm motility

Jenna K Jablonski[1], Olivia D Warren[1], Elizabeth Park[2], J. Matthew Ragle[2], Jordan W Ward[2], Diane C Shakes[11]Biology, William & Mary, [2]Molecular, Cell, & Developmental Biology, UC Santa Cruz

Nematode sperm are crawling cells whose motility is powered by the dynamic polymerization/depolymerization of the nematode Major Sperm Protein (MSP) within their extended pseudopod. Earlier biochemical studies in Ascaris identified MSP polymerization-activating kinase (MPAK) as a key regulator of MSP assembly within the pseudopod. Here we investigate the role of the *C. elegans* MPAK proteins TTBK-8.1 and TTBK- 8.2. The *ttbk* (tau tubulin kinase) genes are conserved across both nematodes and humans. *C. elegans ttbk*-8.1/2 genes are closely related to human *ttbk-1* and *ttbk-2* human genes, which are best known for their involvement in the development of Alzheimer's Disease. As in Ascaris, the *C. elegans* MPAK localizes to the pseudopods of wildtype spermatozoa. Using the power of *C. elegans* genetics, we created single and double knockout mutants. Single knockout males have fertility comparable to wildtype males, while the double knockout males are infertile. *ttbk-8.1/2* males successfully transfer sperm to females, but their sperm fail to crawl to the site of fertilization. During *in vitro* sperm activation, both *ttbk-8.1/2* and wildtype sperm initially extend filopodia-like spikes. Yet compared to wild-type sperm, *ttbk-8.1/2* sperm take three times as long to extend a pseudopod, and they ultimately arrest either as spike intermediates or with extended, non-motile, abnormally smooth pseudopods. Within these pseudopods, MSP localizes normally. Our ongoing studies will characterize TTBK-8.1/2's molecular interactors, and how it regulates sperm motility.

214A A sperm-oocyte protein complex required for egg activation

Tatsuya Tsukamoto[1], Ji Kent Kwah[2], Naomi Courtemanche[3], Katherine M Walstrom[3], David Greenstein[3], Aimee Jaramillo-Lambert[21]ttsukamo@umn.edu, University of Minnesota, [2]Biological Sciences, University of Delaware, [3]Department of Genetics, Cell Biology, and Development, University of Minnesota

In *C. elegans*, SPE-11 is an essential sperm-supplied protein required for egg activation and the oocyte-to-embryo transition. Loss of *spe-11* function results in a paternal-effect failure to produce progeny. We identified a gene, *oops-1*(oocyte partner of SPE-11), encoding a protein that interacts with SPE-11 and localizes to the oocyte cortex. We expressed both proteins in *E. coli* and found that these proteins form a stable complex. Hermaphrodites homozygous for an *oops*-1 null mutation produce no viable progeny, and the defect is specific to the oocyte as *oops-1* null mutant males can sire progeny. Live imaging of oocyte meiosis demonstrated that *spe-11* and *oops-1* null mutants exhibited the same phenotype: approximately half of the fertilized oocytes arrest at either meiosis I or II with the spindle drifting away from the cortex; whereas the rest complete chromosome segregation at meiosis I and II but fail to form polar bodies. The defect in meiotic cytokinesis led us to hypothesize that the OOPS-1–SPE-11 complex might regulate actin assembly. *In vitro* studies showed that the OOPS-1–SPE-11 complex binds F-actin in the absence of other proteins and inhibits formin-mediated actin polymerization. Based on these results, we are currently investigating the role of the OOPS-1–SPE-11 complex in regulating key meiotic F-actin roles such as the formation and stability of the actomyosin contractile ring during meiotic cytokinesis. Lastly, to identify other players that function in the same pathway as the OOPS-1–SPE-11 complex, a large-scale genetic screen for dominant suppressors of a *spe-11* temperature-sensitive mutation was performed. Several suppressing mutations were found in *chs-1, egg-3*, and *gsp-3*. *gsp-3* and its paralog *gsp-4* encode protein phosphatase I subunits that associate with OOPS-1 and SPE-11 in tandem affinity purifications. *chs-1* encodes a chitin synthase required for formation of the chitin layer of the *C. elegans* eggshell. Both *chs-1* and *egg-3* share mutant phenotypes with *spe-11* and *oops-1* (e.g., a failure to form polar bodies). Together, our genetic results support a model in which the OOPS-1–SPE-11 complex interacts with the EGG complex to promote meiotic actin dynamics and eggshell synthesis.

215A The immunoglobulin domain of SISS-1/EGF is critical for its function

Jesse G Jones, Cheryl Van Buskirk Biology, California State University, Northridge

Members of the epidermal growth factor (EGF) family of ligands are found across much of Animalia, including humans, and EGF signaling has been recruited into both behavioral and developmental contexts in vertebrate and invertebrate taxa. Two EGF family ligands have been characterized in *C. elegans*. LIN-3/EGF is critical for several processes including viability, vulval induction, and ovulation. SISS-1/EGF, recently characterized by our group, is essential for stress-induced sleep (SIS). Ubiquitous overexpression (OE) of either ligand can promote a robust sleep state that depends on LET-23/EGFR within sleep-promoting neurons, indicating that they are interchangeable in some contexts. However, SISS-1(OE) promotes much weaker vulval induction compared to LIN-3(OE), even when only the ectodomains are expressed, suggesting that SISS-1 may be a lower-affinity ligand than LIN-3. During our investigation we became curious about the immunoglobulin (Ig) domain present in SISS-1, which LIN-3 lacks. Here, we have present our investigation of the Ig domain of SISS-1, using an endogenous Ig domain deletion as well as transgenic overexpression of Ig-deleted SISS-1. Last, we have added the SISS-1 Ig domain to LIN-3 and examined its effect on LIN-3(OE) phenotypes. Our findings indicate that the Ig domain is critical to both endogenous and ectopic SISS-1 signaling but when attached to LIN-3, it has a weak (or no) effect on its signaling potential.

216A Katanin-mediated severing regulates microtubule patterning during neurite outgrowth.

Dane Kawano, Kang Shen Biology, Stanford

Neurons contain a polarized, staggered, and parallel microtubule array that is important for intracellular transport. However, little is known about how this array is established. Here, we show that in the *C. elegans* PVD neuron, the microtubule severing enzyme, MEI-1/Katanin, is responsible for generating new microtubules during neurite outgrowth. When microtubule severing is impaired, the resulting array contains fewer microtubules, impacting cargo trafficking. Two other proteins, PTRN-1/Camsap and NMTN-1/Wdr-47, play a dual role in recruiting MEI-1/Katanin to the microtubule, and stabilizing the nascent microtubule minus-ends created by MEI-1/Katanin-mediated severing. Together, these proteins amplify microtubule content in the outgrowing neurite in a manner that maintains microtubule polarity.

217B The coupling of global Actin self-organization and endocytosis in the developing *Caenorhabditis elegans* oocyte

Lily Zahwa Khamizan[1], Subhadip Chakraborty[2], Archit Bhatnagar[2], Deborah Sissay[1], Mohammed Faheem[1], Teije Middelkoop[2], Arjun Narayanan[11]New York University Abu Dhabi, [2]Institute of Molecular Genetics, Prague

In *Caenorhabditis elegans*, the actomyosin cortex is first formed as the oocyte develops into an embryo. A major location of actin nucleation during this time is in thousands of transient clusters of WSP-1/WASP, Arp2/3 and F-Actin that continuously appear, grow and fall apart. While the physical chemistry of these clusters has been previously studied, their potential functions have remained mysterious. What are these clusters good for, and how are their dynamics coupled to the rapidly developing first cortex? Here, we describe the discovery that both cluster dynamics and morphology are strongly coupled to global cortical development. Specifically, we find that there are two developmentally cued transitions, the first, co-occurring with nuclear envelope breakdown, involves the emergence of ring-like branched actin structures, and the second co-occurring with RHO-1/RhoA activation, involves the morphological transition of these ring-like structures to punctate clusters. The existence of branched actin rings suggests a connection to endocytosis and their transition to punctate structures as the actomyosin cortex develops highlights a coupling to global actin organization. Indeed, through RNAi directed against actin regulators, we are able to disrupt the transition from rings to puncta. Taken together, our results suggest that the transient branched actin structures formed during early oocyte development are related to clathrin mediated endocytosis and establish the *C. elegans* oocyte as a unique system in which to study the coupling of global actin reorganization, and local endocytic mechano-chemistry.

218B FHOD-1 and profilin collaborate to promote muscle growth and protect sarcomeres from contractile damage in body wall muscle

Michael J Kimmich, David Pruyne Cell and Developmental Biology, SUNY Upstate Medical University

Formins are a class of actin-interacting proteins that nucleate, elongate, and often bundle actin filaments. Nucleation is done through a conserved formin-homology 2 (FH2) domain which binds to actin. Profilin is an actin monomer-binding protein which can interact with the formin-homology 1 domain (FH1) of formins to enhance actin filament elongation. Previously, our lab has found that the formin FHOD-1 in *Caenorhabditis elegans*, is involved in promoting body wall muscle growth. In addition, *fhod-1(-)* null mutants have irregular dense bodies, the Z-line homologs of body wall muscle. We hypothesize that these phenotypes are a result of a loss of FHOD-1-mediated actin filament assembly. In this study, we demonstrate that introducing a point mutation predicted to eliminate actin nucleation activity in FHOD-1 results in identical reduced body wall muscle size and irregular dense bodies to that of a *fhod-1(-)*mutant. Additionally, we discovered that profilin *pfn-2 pfn-3* mutations or a FHOD-1 mutation that eliminates the FH1 domain result in similar irregular dense bodies and smaller body wall muscles. Genetic analysis suggests profilin and FHOD-1 operate in separate and overlapping pathways. Further analysis of *fhod-1* and profilin mutants revealed dense bodies of both were damaged by prolonged contraction and were less stable when compared to wild type animals. Overall, our data suggest that FHOD-1-mediated actin filament assembly is necessary for stable dense bodies, and that profilin also contributes likely through aiding FHOD-1 actin assembly as well as through an additional separate pathway.

219B The role of KFERQ motif in protein degradation

Robert Kinobe[1], Alicia Melendez[2], Sivan Korenblit[3], Solomon Dror[31]Biochemistry PhD Program, The Graduate Center, City University of New York, [2]Biochemistry PhD program, & Molecular Cell Biology, The Graduate Center, City University of New York, Queens College, [3]Life Sciences, Bar-Ilan University

Autophagy ensures that damaged proteins and other cell components are recycled and is a fundamental mechanism to the quality of life of an organism. Autophagy is the natural, conserved degradation of the cell that removes unnecessary or dysfunctional components through a lysosome-dependent regulated mechanism. It requires the sequestration of cargo and the orderly degradation by the lysosome and the recycling of cellular components. Different forms of autophagy are characterized by the mechanism by which the cargo is sequestrated. Macroautophagy (MA) involves the formation of a double-membrane vesicle, the autophagosome, which engulfs the cargo to be degraded and fuses with the lysosome for degradation. Chaperone-mediated autophagy (CMA) is a selective process where specific proteins that contain a pentapeptide KFERQ-like motif are degraded. The KFERQ motif in CMA is recognised by the heat-shock cognate protein of 70 kDa (Hsc70) and the KFERQ containing proteins are transported to the lysosomes and bind to the lysosome-associated membrane protein type 2A (LAMP2A) to be transported into the lysosome. In microautophagy (MI), cargo is degraded when the lysosome or vacuolar membrane invaginates and the target cargo is introduced by pinocytosis. Microautophagy can be endosomal microautophagy (eMI) in which the cargo is delivered to late endosomes to form MVBs for degradation, or into the lysosome. Very little is known about MI in *C. elegans.* It is also unclear what pathways can compensate for impaired macroautophagy. Both eMI and CMA can be selective as they target proteins with the KFERQ motif. However, an evolutionary conserved homolog of LAMP2A is lacking in the genomes of yeast, *C. elegans* and *Drosophila*, suggesting that they lack CMA. Nevertheless, sequence analysis of the *C. elegans* proteome revealed the occurrence of KFERQ-like motifs in about 45% of the proteome. Hence, selective KFERQ-mediated protein degradation could still occur in *C. elegans* through a more ancestral form of MI. Our work, aims to leverage *C. elegans* genetics to investigate the mechanisms involved in the degradation of proteins containing the KFERQ motif in *C. elegans.*

220B Investigating the role of autophagy in stress granule dynamics and proteostasis

Laurel A Koch[1], Caroline Kumsta[2], Malene Hansen[11]Buck Institute for Research on Aging, [2]Sanford Burnham Prebys Medical Discovery Institute

For a cell to survive, it must overcome many different forms of stress; one mechanism to cope with cellular stress is the formation of stress granules (SGs). SGs are non-membranous organelles made up of closely packed mRNAs, translation machinery, and RNA-binding proteins. When stress occurs, translation initiation is stalled, and SGs form protecting and storing non-essential mRNAs during stress. Once the stress is resolved, SGs must be rapidly cleared from the cytoplasm for normal cellular functions to resume. One pathway for clearing SGs is through autophagy, a critical cellular recycling process important for maintaining cellular homeostasis. During autophagy, cargo is enclosed into double-membrane vesicles called autophagosomes, which fuse with hydrolase-containing lysosomes for degradation. Cargos are targeted by selective autophagy receptors, a key example being p62 (SQST-1 in *C. elegans)*, which sequesters ubiquitinated and aggregated proteins. Both p62 and SG have been implicated in the progression of neurodegenerative diseases; however, the role of selective autophagy in SG dynamics remains unclear.

My project seeks to explore the role of selective autophagy in SG dynamics and its contribution to aging and pathological protein aggregation in *C. elegans*. In my preliminary studies, I have found that multiple autophagy genes as well as p62/*sqst-1* are essential for SG clearance in the *C. elegans* germline, supporting my hypothesis that p62 functions as a selective autophagy receptor for SGs. In the future, I plan to explore the role of SGs and autophagy in different tissues throughout aging and in neurodegenerative diseases.

Ultimately, this research aims to elucidate how SGs possibly via p62 affect protein aggregation. Such molecular knowledge will be crucial for advancing our understanding of how SGs and autophagy function is regulated during aging and in neurodegenerative disease, with the ultimate goal to help develop new therapeutic strategies for combatting neurodegeneration and prolonging healthy aging.

221B Cytoplasmic Kinetochore Linear Elements Regulate Vesicle Transport

Shane J Kowaleski, Alexis Bridgewater, Cody Saraceno, Joshua N Bembenek Wayne State University School of Medicine

At fertilization, the oocyte modifies the extracellular matrix after cortical granule exocytosis to prevent polyspermy. In C. elegans oocytes, cortical granule exocytosis occurs in anaphase I and is disrupted after inactivation of a subset of cell cycle genes, including a kinetochore protein. Multiple outer kinetochore components form “linear element” structures in the cortical cytoplasm in oocytes of several species. We found that these kinetochore linear elements begin to form just before NEBD as puncta and grow rapidly. These puncta associate with the endoplasmic reticulum (ER) and microtubules. As the linear elements grow larger, they gather cortical granules surrounded by ER into clusters as part of an elaborate transport mechanism. The kinetochore linear elements dynamically interact with microtubules during and after cortical granule/ER clustering. Tubulin depletion inhibits linear element motility and assembly and inhibits proper cortical granule distribution. Knockdown of Nuf2him-10, a kinetochore protein involved in microtubule plus-end binding, inhibits linear element growth, but not their motility and inhibits cortical granule clustering. Depletion of outer kinetochore components disrupts extracellular matrix formation, which is not observed in inner kinetochore depletion. Therefore, these results indicate that cytoplasmic kinetochore linear elements facilitate the microtubule-dependent transport of cortical granules, which contributes to proper modification of the extracellular matrix. We hypothesize that kinetochore complexes coordinate movements of membrane and chromosomes to enhance the fidelity of cell division.

222B Intercellular glia-glia and glia-neuron interactions regulate AMsh glial apical boundary (GAB)

Alexandra A Kravchuk[1], Sneha Ray[1], Aakanksha Singhvi[1,21]Fred Hutchinson Cancer Center, [2]University of Washington

The nervous system comprises two major cell types, neurons and glia, whose proper interactions support healthy nervous system development and dynamics. Both neuronal and glial functions are underpinned by elaborate, polarized cell morphologies. While we have good insight into how neuronal shapes are regulated, how glia establish and maintain their polarized morphologies remains poorly understood.

To study glial cell polarity, we interrogate the amphid sheath (AMsh) glia of *C. elegans*. AMsh glia exhibit apical-basolateral polarity, with infolded apical membranes contacting amphid neurons and exterior basolateral membranes contacting the amphid socket (AMso) glia and epithelia. We report here our discovery that AMsh glial apical membranes extend a polarized protrusion into the glial process. The termination site of this sub-cellular membrane extension demarcates the posterior-most position of AMsh glia apical membranes. We thus term this structure the glial apical boundary (GAB).

Co-labeling of AMsh glia polarized membranes with tight junction markers confirmed that the GAB is an elaboration of apical membranes and not the AMsh apical-basolateral junction. Further, the GAB is not delimited by neighboring anatomical landmarks. Rather, variation in GAB position between bilateral glia suggests that each AMsh glial cell independently determines its apical boundary. Finally, the GAB is decorated with RAB-11-marked recycling endosomes, the cell adhesion protein HMR-1/Cadherin, and apical membrane (but not cytosolic) tags.

By RNAi and fluorescent expression analyses, we found no role of canonical polarity determinants in regulating GAB architecture. From screens to identify GAB regulators, we instead uncovered roles for homeobox gene ALR-1/Arx, neuronal cilia determinants DAF-19/RFX and DYF-11/Traf3IP1, and scaffolding protein UNC-44/Ankyrin. ALR-1, DAF-19, and DYF-11 are not expressed in AMsh glia, but are in contacting AMso glia, epithelia, or neurons. UNC-44/Ankyrin, meanwhile, expresses broadly. We therefore infer that non-autonomous glia-glia and glia-neuron signaling dictate how individual AMsh glia define their GAB.

In summary, we uncovered that glia have a specialized apical membrane sub-architecture, GAB, regulated by intercellular signaling. We posit that the GAB enables trafficking/sorting of neuron-regulatory cues. Our on-going work focuses on elucidating mechanisms by which these genes regulate AMsh GAB and interrogating GABs of other glial cells.

223B A sexually dimorphic role for HSF-1 in the production of heat-induced DNA damage in the germline

Nicole A Kurhanewicz, Diana E Libuda Biology/IMB, University of Oregon

Maintaining homeostasis and fertility in response to stress is essential for both organism and population survival in a constantly fluctuating environment. Male fertility is particularly sensitive to environmental stress, especially heat stress, with acute heat exposure causing DNA damage specifically in developing sperm (not in oocytes or other tissues) and reduced male fertility across taxa. Exposure to heat stress engages broad and highly conserved mechanisms promoting homeostasis such as the heat shock response (HSR). The HSR is led by master regulator HSF-1, a transcription factor that binds to conserved heat shock elements (HSEs) in the genome. HSF-1 has been implicated in promoting genomic integrity including altering chromatin structure to both activate and repress transcription. In the germline, HSF-1 regulates germ cell maturation, quality control and regulation of transgenerational inheritance after heat stress. Here we identify sexual dimorphisms of HSF-1 and the HSR that regulate the occurrence of heat-induced DNA damage in the *C. elegans* germline. Using immunofluorescence and high-resolution microscopy, we find that during oogenesis, HSF-1 localizes primarily to the nucleoplasm (within the nucleus) and upon acute heat stress HSF-1 associates with oocyte DNA. Conversely during spermatogenesis, HSF-1 largely localizes to the cytoplasm (outside of the nucleus), and upon acute heat stress a fraction of HSF-1 localizes to within the spermatocyte nucleus but remains largely nucleoplasmic and rarely DNA-associated. Moreover, we find that loss of HSF-1 allows the production of heat-induced DNA damage in oocytes, indicating a protective role for HSF-1 in maintaining homeostasis in heat stressed oocytes. Using mRNA sequencing, we identify male-specific upregulation of HSF-1 targets including negative feedback protein Hsp70 in response to heat stress. Taken together, our work finds that the HSR is a sexually dimorphic mechanism underpinning germ-cell specific responses to heat stress that protects the oocyte genome and preserves oocyte fertility.

224B Analysis of cuticle remodeling induced by hypoxia exposure

Mary L Ladage[1], Vijigisha Srivastava[2], Raymond Hendricks[1], Jose Robledo[1], Luhua Song[1], Tamar Malik[2], Einav Gross[2], Pamela Padilla[11]Biology, University of North Texas, [2]Hebrew University of Jerusalem

Skin is a multilayered organ that serves as a protective barrier and communication interface between an animal's interior and the external environment. While the molecular composition of the skin varies widely across taxa, there are many similarities in structure and function such as post-translational crosslinking of cuticle proteins and the presence of a lipid-rich outer layer. The synthesis and post-translation modification of cuticle collagens are both oxygen dependent and it has been established that chronic hypoxia plays a role in the pathology of nonhealing cutaneous diabetic wounds. However, the effects of hypoxia on the composition and function of the cuticle and epicuticle are not fully understood. In this study we used *C. elegans* as a model to examine the impact of hypoxia exposure on cuticle and epicuticle structure and function. We show that hypoxia (0.5% O2) exposure from embryo to the L4 stage induces changes in the L4 cuticle which render it more permeable to small molecules and more resistant to breakage by hypochlorite compared to normoxic (21% O2) controls. This remodeling of the cuticle is modulated by mutation in some, but not all cuticular collagen genes, and requires the function of the lipid transporter *gmap-1*. Next, to understand the transcriptomic changes induced by hypoxia or by *gmap-1* mutation, we performed RNA-sequencing on wild-type (N2) and *gmap-1(ulb13)* mutant animals grown in normoxia or hypoxia from embryo to the late L4 stage. Bioinformatic analysis shows that the transcriptomic profiles of animals raised in hypoxia are distinct from their normoxic counterparts, and wild-type animals and *gmap-1(ulb13)* mutants have distinct profiles. GO term analysis indicates strong enrichment of GO terms associated with molting cycle, collagen and cuticulin-based cuticle in wild-type animals and to a lesser extent in *gmap-1(ulb13)* animals grown in hypoxia. To further understand the role of *gmap-1*, we generated a *gmap-1::mKate* translational reporter using a CRISPR editing approach. Initial analysis indicates that *gmap-1* is expressed in the developing vulva lumen as well as in the cuticle and epidermis. Future directions include detailed analysis of this and of other collagen reporters and a lipidomic approach to understand changes to the epicuticle induced by hypoxia. These results demonstrate that hypoxia induced cuticle changes can be modulated genetically and provide pathways to investigate with respect to nonhealing wounds.

225B Challenges in Humanising *C. elegans*: Insights from the ciliary transition zone protein MSKR-2/B9D2

Karen I Lange University College Dublin

The transition zone is a distinct region at the base of cilia that acts as diffusion barrier for membrane and cytoplasm. The transition zone is essential to maintain the unique molecular composition of the cilium and mutations in transition zone genes cause rare genetic disorders called ciliopathies. Cilia and transition zone biology are remarkably conserved between humans and worms; many human transition zone proteins have clear orthologues that are functionally conserved in worms. Using CRISPR-Cas9 genome editing, patient variants can be modelled in endogenous worm genes. These disease models allow us to better understand how mutations in these genes cause ciliopathies. However, with this approach, only conserved amino acids can be targeted. This limitation can be addressed by "humanising" the worms: replacing the endogenous *C. elegans* gene with human cDNA codon-optimised for expression in worms. I selected *B9D2*/*mksr-2* for this project because it is the most highly conserved transition zone protein (63.4% amino acid similarity), it is relatively small (175 aa) with only 4 exons, and there is an mNeonGreen::*mksr-2* knock-in strain available to assess protein localisation. Using CRISPR genome editing, I replaced the endogenous *mksr-2* coding region with codon-optimised human B9D2 with three synthetic introns (CeB9D2). I found that mNG::CeB9D2 fluorescent signal is not detectable and is indistinguishable from a null allele. To determine if the humanised gene was being expressed, I amplified full length cDNA. Unexpectedly, this generated multiple PCR products larger than anticipated, suggesting abnormal splicing. This result was surprising because the construct contained standard synthetic introns which are typically robustly spliced. To address this, I am in the process of generating two new humanised CeB9D2 strains: one that retains endogenous introns and one with no introns. These experiments are on-going, but preliminary data is not optimistic. Simply put, this gene may not be compatible with humanisation. MKSR-2/B9D2 is part of a heterotrimeric complex (MKS1/B9D2/B9D1) and the extensive protein-protein interactions in the complex may not be compatible with humanisation of only one subunit. I propose that proteins which are known to make extensive protein-protein interactions may not be optimal targets for humanisation.

226B Control of ciliary extracellular vesicles biogenesis in *C. elegans*

Patrick Laurent ULB Neuroscience Institute, Université Libre de Bruxelles

Extracellular vesicle release from cilia is conserved across species, yet its biogenesis and function remain poorly understood. In *C. elegans*, we previously observed the outward budding of ciliary ectosomes from sensory neurons, carrying intraflagellar transport (IFT) proteins and membrane receptors transported by IFT. This process may prevent receptor accumulation when IFT is disrupted. We established TSP-6::wSc as a reliable ciliary membrane marker that moves by diffusion rather than IFT, allowing the unbiased quantification of ectosome release and the mapping of all potential budding sites. Under normal conditions, ectosome release is rare but increases significantly with acute sensory stimuli. All known mutants disrupting receptor trafficking within cilia also show elevated ectosome release. Facilitating receptor entry the ectosomes, the budding sites vary among mutants: at the ciliary tip (*mks-6*), along the cilium (*bbs-8*), in the proximal cilium (*arl-13*), and at the periciliary membrane compartment (PCMC) (*tub-1* and IFT-disrupting mutants). Notably, basal or apical ectosome release correlates with increased or decreased PCMC size, suggesting PCMC dynamics regulate ectosome budding. Our findings demonstrate that disrupted membrane protein trafficking and sensory signaling promote ciliary ectosome biogenesis at specific locations along the cilia. This supports the hypothesis that ectosome shedding serves as a homeostatic mechanism to prevent local receptor accumulation and to regulate ciliary signaling.

227B Identification of Gene Regulators of Germline Development in *C.elegans*

Sheldon R Lawrence[1], Brandon Brewer[21]Biology, Oxford College of Emory University, [2]Emory University

The regulation of stem cell proliferation vs. differentiation lies at the heart of many biological and biomedical problems ranging from fetal development to tissue engineering to aging and death. The specification of the germline stem cells, the robust maintenance of this population, and its differentiated descendants that form gametes are essential to an organism's function. We study cell signaling pathways that regulate stem cells and the germline stem cell niche in the nematode worm *C.elegans*. Our current research seeks to address a key question in stem cell biology about how the balance between stem-like and differentiating cell fates is maintained and regulated. In the present study, to identify genes that affect gonadal development, worms were synchronized at the L1 larva stage, and bacterial-mediated RNA interference (RNAi) treatment was used to knock down three genes (Jun-1, Xtr-2, and Mef-2). Post 48-hour RNAi treatment, fluorescent microscopy was used to analyze gonadal morphology, including length and width, and a student's T-test was performed to determine statistical significance. Our findings concluded significant differences between the mean length/width of experimental groups jun-1, xtr-2, and mef-2 (RNAi) and that of the RNAi-negative control vector L4440. This evidence suggests that the genes may be necessary for gonad development and/or maintenance. The significance of this finding allows us to begin validating our candidate genes and constructing a biological gene-interaction/protein-protein interaction pathway map, which will help us better understand signaling pathways involved in germline development and maintenance.

228B Y45G5AL.1 is a potential kinesin-1 interacting protein on the surface of yolk granules during meiotic spindle translocation

Denisa G Lazureanu, Francis J McNally Molecular and Cellular Biology, University of California Davis

Female meiosis is a process by which the genetic material segregates to produce one haploid oocyte and two polar bodies. Improper polar body extrusion can cause aneuploidy/polyploidy in the oocyte, leading to genetic abnormalities or embryonic lethality. Meiotic spindle positioning at the cell cortex is essential for proper genetic material segregation into polar bodies in mammals. In *C.elegans*, the motor protein kinesin-1 is required for early spindle translocation to the cortex during meiosis I metaphase. Kinesin-1 is thought to push the spindle outwards during ovulation by packing organelles inwards along microtubules with minus ends oriented at the oocyte cortex. Constitutively active kinesin-1 hinge-tail constructs prematurely pack yolk granules into a tight ball in immature oocytes. This result suggests that yolk granules are a direct cargo of kinesin-1. Kinesin cargo adaptor proteins act by binding to organelle-specific membrane proteins and attaching them to kinesin for transport. KCA-1 is a cargo adaptor necessary for kinesin-1 packing of yolk granules, although the KCA-1 associated yolk granule-specific membrane protein is unknown. A BioID experiment using KCA-1 as bait identified the protein Y45G5AL.1 as one potential target. To understand whether Y45G5AL.1 is a genuine KCA-1 interacting protein on yolk granules, we tested for co-localization with KCA-1 and inward packing of Y45G5AL.1 in a constitutively active kinesin-1 strain. We prepared a strain with KCA-1::GFP and Y45G5AL.1::HALO in a wild-type kinesin-1 background and left the strain on *rab-7* RNAi for 24 hours to increase yolk granule size. Through fluorescence microscopy imaging, we found that Y45G5AL.1 and KCA-1 appear in rings around yolk vesicles and demonstrate co-localization. We also prepared a strain with KCA-1::GFP and Y45G5AL.1::HALO in a hinge-tail kinesin-1 background on *rab-7* RNAi for 24 hours. Fluorescence microscopy revealed a mass of Y45G5AL.1 and KCA-1 packed into the center of immature oocytes. The packing of Y45G5AL.1 and KCA-1 suggests they are both present on kinesin-1 cargos. For future experiments, we plan to test the role of Y45G5AL.1 in normal yolk granule packing and meiotic spindle movement to the cortex.

229B Endogenous expression patterns of canonical cell-death genes

Dongyeop Lee, Bob Horvitz HHMI/MIT

Programmed cell death is an evolutionarily conserved process essential for development and tissue homeostasis in metazoans. The process of cell death is regulated by four core cell-death genes: *egl-1* (BH3-only), *ced-9* (BCL-2), *ced-4* (APAF-1), and *ced-3* (caspase). In cells fated to die, EGL-1 is expressed and binds to CED-9 at mitochondria, inducing a conformational change that releases CED-4 from CED-9. Freed CED-4 forms octamers and activates the caspase CED-3 to execute cell death. While genetic, biochemical and structural studies have extensively characterized these interactions, the spatial and temporal expression patterns of these genes and the localization of their protein products during development remain incompletely understood, in part because of the limitations of traditional transgenes, which can miss regulatory elements or result in overexpression artifacts. To address these challenges, we used CRISPR/Cas9 to generate fluorescent translational reporters for the four canonical cell-death genes expressed from their endogenous loci: *GFP::H2B::F2A::egl-1* (*GFP::egl-1*), *wrmScarlet::ced-9*, *ced-4::GFP*, and *ced-3::wrmScarlet*. We observed that, as expected, *GFP::egl-1*-positive cells accumulate in cell-death-defective mutant embryos and larvae. We confirmed that *GFP::egl-1* expression is detected specifically in the undead sisters in the cell-death mutants but is absent in their surviving counterparts, indicating that *GFP::egl-1* serves as a robust marker for identifying undead cells. We also confirmed that wrmScarlet::CED-9 and CED-4::GFP localize to mitochondria and that in the absence of *ced-9*, CED-4::GFP is located at the perinuclear membrane. In the adult germ line, CED-4::GFP is enriched at the perinuclear membrane and loss of *ced-9* function further enriches CED-4::GFP at the perinuclear membrane, suggesting that CED-4 is expressed at a higher level in the adult germ line than in other tissues. Interestingly, CED-3::wrmScarlet is enriched in the nuclei of early embryos and adult germ cells. We also found that both CED-4::GFP and CED-3::wrmScarlet accumulate in cells undergoing cell death during embryogenesis. Using these reporters, we are currently performing single-cell RNA sequencing studies of undead cells and proteomic analyses of CED-4-binding proteins. Our work provides new insights into the dynamic regulation of cell-death genes in living animals and establishes valuable tools for dissecting the molecular mechanisms of cell death.

230B Multi-copy FLP Recombinase Expression Disrupts Glutamate Receptor Trafficking in *C. elegans*

Zephyr Lenninger, Frederic Hoerndli Colorado State University

Fluorescent imaging of cellular processes requires precise strategies to enable spatial control of fluorescent tags. DNA recombinases such as Cre and FLP allow for efficient, cell-specific fluorescent labeling of endogenous proteins. As the popularity of these systems increase, so do the reports of off-target effects and cytotoxicity due to overexpression. Cre overexpression specifically results in DNA damage, so there has been a shift towards the FLP/FRT system. Yet, there is still motivation to express multiple copies of FLP in driver lines to ensure a complete gene edit. In *C. elegans*, the FLP/FRT system has been utilized for over a decade. The two most prominent approaches to express a FLP recombinase in *C. elegans* are a) single-copy integration, or b) multi-copy expression from an extrachromosomal array. Our lab specializes in *in vivo* imaging of glutamate receptor (GLR-1) trafficking in the command interneurons AVA, and we are interested in utilizing the FLP/FRT system to label endogenous synaptic markers. During the initial trials, we observed that multi-copy expression of a FLP recombinase results in a GLR-1 trafficking deficit. We sought to determine how FLP expression affects GLR-1 trafficking and if the deficit is dose-dependent. We generated multiple lines of animals with distinct extrachromosomal arrays that express FLP in AVA as well as a single copy integration of the same FLP plasmid. In animals expressing multiple copies of FLP, we discovered a significant decrease in GLR-1 expression, transport velocity, and a 50% decrease in transport events when compared to same-plate animals that lack the extrachromosomal array. The trafficking deficit persists in the absence of FRT sites, suggesting that the effect is distinct from the targeted activity of FLP. This decrease in transport is not present in animals expressing a single-copy integration of the same plasmid. We conclude that the FLP recombinase is indirectly affecting GLR-1 trafficking by inhibiting glutamate receptor expression in a dose-dependent manner.

231B Centrosome Maturation Inhibition during *C.elegans* Female Meiosis

Wenzhe Li, Frank McNally Molecular and Cellular Biology, UC Davis

In most animal species, fertilization occurs during female meiosis (prometaphase in *C.elegans*), and centrioles are eliminated early during oogenesis and reintroduced by sperm during fertilization. However, sperm-derived centrioles don't mature into centrosomes until female meiosis is complete. Centrosome maturation requires the expansion of pericentriolar material (PCM), a process typically initiated by recruiting PCM scaffold protein to the centrioles. Our lab previously discovered that in *C.elegans*, the recruitment of maternal PCM scaffold protein SPD-5 to sperm derived centrioles is completely suppressed after fertilization throughout female meiosis, from prometaphase I through anaphase II, by the kinesin-1 cargo adaptor protein KCA-1. However, the mechanism through which the inhibition happens remains unknown. Here, using auxin induced degradation system specific for *C.elegans* germline, we discovered that KCA-1, kinesin-1 light chains KLC-1 and 2, and kinesin-1 heavy chain (UNC-116) all spatially and temporally control SPD-5's localizations around the sperm-derived centrioles in the zygote. In the absence of KCA-1or kinesin-1 subunits, SPD-5 localizes onto the sperm derived centrioles before female meiosis completes, and forms extra acentriolar foci near the centrioles. To understand how KCA-1 suppresses centrosome maturation, we conducted Turbo-ID experiments with KCA-1 as bait and identified SPD-5 as one of the most enriched KCA-1 interactors. To validate this potential in vivo interaction between KCA-1 and SPD-5, we monitored SPD-5 localization in immature oocytes expressing a constitutively active kinesin-1 heavy chain, which compacts kinesin-1 direct cargos into a tight ball in these oocytes. Expression of constitutively active kinesin-1 prematurely packed SPD-5 inward into a tight ball in immature oocytes, suggesting that the inhibition of SPD-5 by KCA-1/kinesin-1 is due to a direct interaction between them. Surprisingly, artificially recruiting GFP::SPD-5 into close proximity with the sperm centrioles was not sufficient to drive premature centrosome maturation. The centriole-localizing SPD-5 network expanded more efficiently than SPD-5 ectopically recruited to paternal DNA in KCA-1 depleted zygotes, suggesting the importance of additional proteins at centrioles in facilitating centrosome maturation. We are currently exploring the mechanism through which the direct interactions of KCA-1/kinesin-1 and SPD-5 lead to centrosome maturation inhibition during *C.elegans* female meiosis.

232B Understanding Axonal mRNA Localization Mechanism in *C. elegans*

Hui Yuan Lim, Chaogu Zheng School of Biological Sciences, The University of Hong Kong

Axons extend over long distances from the soma to nerve endings and rely on localized mRNA translation to regulate synapses and rapidly respond to extracellular cues. We are investigating the axonal localization mechanism of *mec-17* mRNAs, which encode a tubulin acetyltransferase, in the touch receptor neurons (TRNs) of *Caenorhabditis elegans*. Using single-molecule fluorescence *in situ* hybridization (smFISH), we found that the *mec-17* mRNA is trafficked to the axons of the TRNs and that the *mec-17* mRNA abundance decreases from the cell body proximal region toward the distal ends of axons. To study *mec-17* mRNA dynamics in living animals, we developed a visualization tool based on the MS2/MCP system. We inserted 24xMS2 to the 3'UTR of *mec-17* and expressed a MCP::GFP fusion protein specifically in the TRNs. We confirmed that the GFP signal colocalizes with *mec-17* smFISH signal, suggesting that the labelling is reliable. Using this strain, we are able to track the dynamics of the *mec-17* mRNA molecules in living neurons and found bidirectional movement of the mRNA in axons, as well as long-distance travel. By expressing truncated *mec-17* mRNAs in the *mec-17(-)* mutants and conduct smFISH on the transgenic animals, we identified a minimally essential 60-bp region in the 3'UTR of *mec-17* mRNA, which is required for *mec-17* mRNA stability, but neither this element nor the whole *mec-17* 3'UTR is sufficient to drive axonal trafficking of other mRNAs. We then searched for potential *cis*-regulatory element in the coding region of *mec-17*. We expressed a recoded version of *mec-17* mRNA, in which the codons were replaced by synonymous codons to disrupt potential *cis*-regulatory RNA sequences without affecting the amino acid sequence, in *mec-17(-)* mutants and conducted smFISH against the recoded *mec-17*. To our surprise, the recoded *mec-17* mRNA can still travel to the axons, suggesting that the *mec-17* mRNA transport is translation-dependent. In fact, inhibiting translation using puromycin was able to disrupt the axonal transport of *mec-17* mRNAs. We are in the process of analyzing the coding sequence of MEC-17 to identify the protein domain or region required for the transport. Currently, we are setting up a genetic screen to identify the factors that regulate the *mec-17* mRNA trafficking. One of the candidate we are testing is UNC-104, the *C. elegans* homolog of human KIF1A (kinesin family member 1A).Given the link between mRNA trafficking dysfunction and numerous diseases, our findings may provide insights into the pathological mechanisms underlying neurodevelopmental disorders and neurodegenerative diseases.

233B Tissue-Specific perinuclear localization of the APAF-1 homolog CED-4

Mona H.X. Liu[1,2], Dongyeop Lee[2], H. Robert Horvitz[1,21]Harvard Medical School, [2]Biology, Howard Hughes Medical Institute, Massachusetts Institute of Technology

Apoptosis is a highly regulated and evolutionarily conserved process that eliminates unwanted cells. In *C. elegans*, the subcellular localization of the pro-apoptotic APAF-1 homolog CED-4 has been thought to control the cell-death fate: sequestration of CED-4 to mitochondria by the BCL-2 homolog CED-9 inhibits apoptosis, whereas oligomerization and translocation of CED-4 to the nuclear periphery promotes apoptosis. However, recent findings from our laboratory (Tucker *et al*., Sci. Adv. 10, eadn0325, 2024) have challenged this canonical model — CED-9/CED-4 interaction at mitochondria is required for the non-canonical pro-apoptotic function of *ced-9*, rather than for its canonical anti-apoptotic function. In addition, we and others have observed that CED-4 is localized to the nuclear periphery of healthy syncytial germ cells and oocytes that are not undergoing apoptosis. The subcellular localization of CED-4 thus appears to play a nuanced and incompletely understood role in apoptosis and possibly other processes. To determine the mechanism responsible for the perinuclear germline localization of CED-4, we used a CRISPR/Cas9-generated strain that expresses a CED-4::GFP fusion protein (see abstract by Lee and Horvitz) and performed a genetic screen for mutants altered in the localization or expression pattern of the CED-4::GFP protein. So far, we have isolated two mutants. One causes some CED-4::GFP expression to radiate outward from its normally smooth ring-like pattern at the nuclear periphery. The other mutant induces a ring-like pattern of CED-4::GFP to form ectopically around hypodermal nuclei, with the brightest expression around a set of lateral nuclei (possibly seam cells) anterior to the vulva in the adult hermaphrodite. We are currently characterizing these mutants and planning to use RNA interference to test candidate genes encoding nuclear envelope proteins to identify additional factors that mediate the perinuclear localization of CED-4. We hope to gain insights into how and why the subcellular localization of CED-4 is regulated in a tissue-specific manner to control apoptosis and possibly other processes that contribute to development and tissue homeostasis.

234B Genes Needed for Benomyl Resistance and Hypersensitivity in *Caenorhabditis elegans*

Zhenhao Guo, Sophie Aminololama López, Martin Chalfie Biological Science, Columbia University

Benzimidazole anthelmintics, such as benomyl, paralyze *C. elegans* and slow their growth by targeting the BEN-1 β-tubulin. Loss of *ben-1* results in normal-appearing animals that are completely resistant to benomyl and other benzimidazoles (Driscoll et al., 1989), a result suggesting that BEN-1 acts redundantly in *C. elegans*. Since 74% of benzimidazole-resistant *C. elegans* strains isolated from the wild had *ben-1* mutations (Hahnel et al. 2018), defects in other genes must also convey resistance. To identify such genes, we screened 1,751 Million Mutation Project strains. We found two completely resistant strains (both with *ben-1* mutations), 14 partially resistant strains (in which animals were mobile but uncoordinated on benomyl), and five strains with increased sensitivity (wherein animals were paralyzed at a low, normally ineffective benomyl concentration). We identified the causative mutations in five partially resistant strains, all in different genes: *ben-1*, the microtubule depolymerase gene *klp-7*/kinesin-13, and three genes expressed in ciliated sensory neurons (the intraflagellar transport particle genes *osm-1*/IFT-172, *che-13*/IFT57 and *dyf-3*/IFT38). In addition, we found that mutations of *daf-19* (in the presence of a *daf-12* mutation to block dauer formation), which encodes a key transcription factor for ciliogenesis, and 17 other cilia-related genes also produced partial resistance. A similar result for 12 of the latter genes was reported in a preprint in BioRxiv by Robert A. Brinzer et al. in 2021. This finding suggests that benomyl is transported into *C. elegans* via ciliated neurons as Brinzer et al. (2024) found for macrocyclic lactones. These results together suggest that ciliated neurons may be important conduits for entry of diverse exogenous molecules into *C. elegans*. Three of the five strains with increased sensitivity had mutations in *tbb-2*/β-tubulin (a result consistent with that of Pallotto et al. 2022). A fourth, similarly sensitive strain had a mutation in *tba-1*/α-tubulin. These results and the partial resistance caused by KLP-7 suggest that microtubule stability may affect the strength of the drug's effect, perhaps by changing the inherent stability of the microtubules or the amount of BEN-1 incorporated in them.

235B *C. elegans* IGEG-2 is a functional EGFR ligand

Melissa M Mailhot[1], Darlene Mendez[2], Jesse Jones[2], Cheryl Van Buskirk[21]Biology, California State University, Northridge, [2]California State University, Northridge

Epidermal Growth Factor (EGF) signaling is used by animals to regulate events from cell proliferation to sleep, and its dysregulation in humans is associated with cancer. *C. elegans* possesses one known EGF receptor, LET-23/EGFR, and two known EGFR ligands LIN-3 and SISS-1. LIN-3 specifies cell fates during several developmental events, most notably during vulval induction, and SISS-1 mediates stress induced sleep (SIS) via activation of EGFR within sleep-promoting neurons. Our lab is currently characterizing a transmembrane EGF domain containing protein, IGEG-2, as another potential EGFR ligand. Importantly, the *igeg-2* gene contains an intron between the fourth and fifth cysteines in the EGF motif, a near-invariant feature of EGF family ligands. The EGF domain of IGEG-2 shares no more sequence identity with LIN-3 or SISS-1 than with vertebrate EGFs, indicating that *igeg-2* did not arise by recent gene duplication. Using transgenic overexpression (OE), we show that IGEG-2 can activate known LET-23-dependent events such as vulva induction and sleep. Like SISS-1, the IGEG-2 ectodomain contains an immunoglobulin (Ig) domain, which we have found to be critical for IGEG-2(OE) phenotypes. As *igeg-2* null mutants are superficially wild type, the endogenous function of IGEG-2 is still under investigation.

236B Characterization of the Receptor Accumulation and Degradation in the Absence of Recycling (RADAR) pathway in *C. elegans*

Kwangsek Jung[1], Pak Yin Wong[1], Jimmy H Mo[1], Ho Yi Mak[21]Hong Kong University of Science and Technology, [2]Division of Life Science, Hong Kong University of Science and Technology

Peroxisomes are conserved eukaryotic organelles that are solely responsible for the processing of specific classes of fatty acids including very long chain fatty acids. Defects in peroxisomal biogenesis or function cause severe phenotypes, including neonatal death, in human patients with mutations in the peroxin (PEX) genes. Peroxisomal function is critically dependent on the import of matrix proteins by the conserved transporter PEX5, which shuttles between the cytoplasm and peroxisomes. The PEX5 level can also be regulated by the Receptor Accumulation and Degradation in the Absence of Recycling (RADAR) pathway when recycling is impaired. It has been shown that PEX5 recycling or degradation depends on its passage through the PEX2/PEX10/PEX12 retrotranslocation channel. In addition, PEX2-dependent mono-ubiquitination or PEX10-dependent poly-ubiquitination is required for PEX5 recycling or degradation, respectively. So far, results that support the PEX2- or PEX10-specific function were largely derived from reporter assays using an artificial peroxisomal matrix cargo in yeast. Here, we use *C. elegans* as a metazoan model to link peroxisomal protein import with organismal physiology. Using CRISPR, we have introduced knockin mutations in *C. elegans* orthologs of PEX2 or PEX10 that specifically eliminated their ability to ubiquitinate PEX5, while maintaining the integrity of the retrotranslocation channel. The import of a fluorescent protein tagged-peroxisomal cargo, expressed at the endogenous level, was compared between wild type and mutant strains. Our results revealed tissue-specific perturbation of cargo import when PEX5 degradation was impaired. Our work has implications on the molecular basis for tissue-specific vulnerability in patients with peroxisomal disorders.

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237B Dual screens of a *C. elegans* Joubert Syndrome model

Anastasios Mazarakis, Karen Lange University College Dublin

Primary cilia are microtubule-based organelles with important signal transmission and sensory functions. Ciliopathies are a heterogenous group of about 35 rare diseases caused by dysfunctional cilia, presenting overlapping symptoms on most organs and a varying degree of severity. Joubert syndrome is a neurodevelopmental ciliopathy with developmental delay and multiple congenital anomalies, characterized by a midbrain hindbrain malformation. There are currently no treatments for patients with Joubert Syndrome. Many cilia proteins are functionally conserved between *C. elegans* and humans, so worms can be used to model patient variants. This project focuses on a pathogenic missense allele of B9D2 (P74S) that was identified in a patient with Joubert Syndrome and has previously modelled in the *C. elegans* ortholog, *mksr-2*. In an *nphp-4* background, *mksr-2(P74S)* worms exhibit several cilia defective phenotypes including dye filling, osmotic avoidance, and roaming defects. This project aims to use two parallel screens to identify novel therapeutic targets for Joubert Syndrome. Both screens use the dye filling assay to indirectly assess cilia integrity assay. The first screen is an EMS mutagenesis screen to identify genetic suppressors of *mksr-2(P74S); nphp-4*. The second screen is a drug screen of small molecules that improve cilia integrity in the *mksr-2(P74S); nphp-4* worms. The goal of this project is to use these complementary screens to identify cellular components and pathways that can be modulated to restore cilia function in Joubert Syndrome models. Ultimately, we hope to identify novel treatments for patients with Joubert Syndrome.

238B Modulating activities and/or targeting of protein kinases during meiosis by protein phosphatase complexes

Savannah McCoy, Baptiste Roelens, Celja Eubel, Anne Villeneuve Developmental Biology, Stanford University

Multiple events that must occur in meiotic prophase to ensure proper chromosome segregation are coordinated by dynamic temporal and spatial regulation of the phosphorylation states of key meiotic proteins. Protein kinases central to meiosis include CHK-2, which is activated at prophase onset, and Polo-like kinase PLK-2, which dynamically associates with distinct nuclear structures at different stages of prophase via primed phosphorylation of polo-binding motifs (PBMs). We are investigating how distinct protein phosphatase complexes may regulate or counterbalance activities of these protein kinases.

We were prompted to investigate meiotic roles of Protein Phosphatase 4 complexes containing the PP4R3 regulatory subunit SMK-1 based on parallels with the nucleoplasmic HAL-2/HAL-3 complex, previously implicated in constraining PLK-2 activity to coordinate meiotic events. SMK-1 similarly localizes in the nucleoplasm, and loss of SMK-1 or the PPH-4 catalytic subunits phenocopies hallmark hal-2/hal-3 phenotypes including: 1) failure to localize PLK-2 to nuclear-envelope attachment sites called pairing centers (PCs) that mediate early-prophase chromosome movements, and 2) inappropriate association of synaptonemal complex (SC) subunits (SYP proteins) with unpaired chromosome axes. However, CHK-2 activity is not diminished, indicating distinct roles for SMK-1/PP4 and HAL-2/HAL-3 complexes. Mutating a predicted PP4 docking site (syp-1(F444A)) near the known SYP-1 T452 PBM reduces the number of PLK-2 PC foci, supporting a role for PP4 in counteracting phosphorylation of “decoy targets” that can antagonize PC localization of PLK-2 in early prophase. Unexpectedly, enrichment of PLK-2 on SC sub-domains during late prophase is diminished in the syp-1(F444A) mutant despite phosphorylation of the SYP-1 T452 PBM, and other aspects of late-prophase SC and chromosome axis differentiation are also successful. This finding suggests that while the ability of PLK-2 to interact with primed targets may be important for later prophase differentiation of meiotic bivalents, localized enrichment of PLK-2 on SCs is dispensable, implying substantial redundancy in the mechanisms that ensure proper bivalent differentiation to drive correct segregation.

We will also report on our work investigating the meiotic roles of ZK688.9, a conserved predicted regulator of Protein Phosphatase 2A, identified as a component of the meiotic machinery in our recent Green eggs & Him 2.0 genetic screen.

239B Identification of new players regulating uniparental mitochondria inheritance

Valentine Melin, Justine Cailloce, Fanny Husson, Romina Cerjani, Vincent Galy, Jorge Merlet Sorbonne Université, CNRS, INSERM, Development, Adaptation and Ageing, Dev2A

Mitochondria are essential organelles in eukaryotic cells; they provide cellular energy and are also required for many cellular pathways. Mitochondria contain their own genome (the mtDNA), which encodes only a small fraction of a cell's genes, but is nonetheless essential. During sexual reproduction, and unlike the nuclear genome, which comes equally from both parents, the mitochondrial genome is inherited only from the mother. In fact, uniparental maternal mitochondria inheritance is the most common form of mtDNA transmission in the animal kingdom and is also found in the nematode *C. elegans*. Although not inherited, sperm mitochondria enter the oocyte during fertilization and are actively degraded by a specific mechanism of mitophagy called *allophagy*. This implies a highly selective recognition mechanism. Despite recent significant progress in identifying some of the factors involved in the degradation processes, the signals carried by sperm mitochondria that trigger their specific recognition and degradation in the embryo remain largely elusive. To identify such mark(s), we used combinations of proteomic and a gene candidate approaches to test the involvement of conserved mitochondrial proteins in uniparental mitochondria inheritance. I will present this work, which led to the identification of novel factors of the *allophagic* pathway and our exploration of their function(s) in uniparental maternal mitochondria inheritance.

240B A high-glucose diet decreases fertility in male *C. elegans* by reducing sperm size, competitiveness, and quantity

Kerry A Larkin, Jason Erichsen, Marjorie R Liggett, David Chen, Goniola Bode, Hannah E Costa, Caroline D Davis, Jose Garcia-Chope, Michael Mastroianni, Bethany Weinberg, Shuqi Yun, Michelle A Mondoux Biology, College of the Holy Cross

Infertility affects ~15% of Americans of reproductive age. High-sugar diets, obesity, and type 2 diabetes have all been associated with infertility, and have been correlated with decreases in sperm and oocyte quality and viability. Despite the burden of infertility and the prevalence of high-sugar diets, the cellular and molecular mechanisms that link diet to fertility are unknown.

As in humans, a high-glucose diet leads to reduced fertility in *C. elegans*. Previously, our lab and others have shown that a high-glucose diet reduces hermaphrodite self-fertility. We found that high-glucose diet also reduced male fertility in a dose-dependent manner. Concentrations of glucose that have no effect on hermaphrodite self-fertility disrupted mated fertility, which allowed us to separate the effects of a high-glucose diet on males from its effects on hermaphrodites.

Fertility is a complex phenotype, and a high-glucose diet could disrupt one or more mechanisms. We tested several aspects of male fertilization success and found that high-glucose diet reduced both the quantity and quality of male sperm. We found that high-glucose diet reduced male sperm count. In addition to producing fewer sperm, the sperm produced by males on a high-glucose diet were also less competitive. On a control diet, male sperm is used almost exclusively when males are mated to hermaphrodites. However, on a high-glucose diet, we found a reduction in the percentage of offspring derived from male sperm, and this reduction in male sperm competitiveness suggests a reduction in sperm quality. When males were crossed to *fog-2* "females" that cannot produce their own sperm, the number of live offspring produced was lower than the number of live offspring produced in a hermaphrodite cross, suggesting that in addition to a decrease in competitiveness, high-glucose diet led to a decrease in competence, or the ability of the sperm to fertilize an embryo that is then capable of development.

Several facets of sperm biology are known to correlate with quality and competence, most notably size. We found that high-glucose diet led to a significant decrease in spermatid size and a significant decrease in embryo viability. We found no effect of high-glucose diet on spermatid morphology or activation, and small or no effects on male mating success, mating behavior, or sperm transfer during mating.

Understanding how a high-glucose diet affects male gametes contributes to our understanding of how diet affects fertility in *C. elegans* and can provide insight into the range of cell biological responses to excess glucose.

241B Reversible aggregation of nucleoporins under extreme heat shock

Paula Monterrubio Asensio[1], Cristina Ayuso García[2], Rafael Rodríguez Daga[2], Peter Askjaer[11]Centro Andaluz de Biología del Desarrollo, Andalusion Centre for Developmental Biology, [2]Andalusion Centre for Developmental Biology

Nuclear pore complexes (NPCs) are composed of multiple copies of 30-35 nucleoporins (Nups; NPPs in *C. elegans*) embedded in the nuclear envelope and act as regulators of RNA and protein transport between the cytoplasm and nucleoplasm. In addition to their function in nucleocytoplasmic transport, the contact of Nups with chromatin at NPCs and in the nuclear interior has allowed the emergency of non-transport functions. These non-canonical functions of Nups are relevant to human pathologies and developmental phenotypes that arise as a result of Nup mutations. Moreover, there are Nups that gain a new localization upon certain stress conditions, such as heat stress. These "mobile Nups" have been detected in human, nematodes and fission yeast cells. The mechanisms underlying the mobility of Nups, and the phenotypes and pathologies associated with defects in these new roles of mobile Nups are just emerging in the field. We hypothesize that specific nucleoporins have acquired new functions during evolution, acting therefore as moonlighting proteins. We recently discovered a novel heat stress-induced structure in fission yeast, termed nucleolar rings (NuRs), which accumulate of a specific subset of Nups (PMID 33176152). Moreover, ectopic expression of NPP-10N/Nup98 can lead to cytoplasmic Nup aggregation and paralysis (PMID 37254647). To uncover moonlighting functions of nucleoporins conserved across species we apply acute heat stress to a variety of *C. elegans* strains with deletions and/or fluorescent tags in endogenous Nup (*npp*) loci.

We report that the Nups that form the nuclear basket structure of the NPC (NPP-7/Nup153, NPP-16/Nup50 and NPP-21/TPR) detach rapidly from NPCs upon exposure to heat stress whereas most other Nups remain stable. Nuclear protein import is perturbed but still active under these conditions. The aggregation of detached Nups in nucleoplasmic foci depends on heat shock transcription factor 1 (*hsf-1*), suggesting that the foci are part of a reversible physiological stress response that remove the nuclear basket of the NPC.

242B Assessing heat-induced DNA damage in H3K9 methyltransferase mutants during *C. elegans* spermatogenesis

Daela B Montgomery[1], Hannah R Wilson[2], Diana Libuda[21]Biology, University of Oregon, [2]University of Oregon

The production of sperm and eggs through meiosis is essential for reproduction and proper passage of the genome. Across many species, including C. elegans, heat-stress impacts the quality of sperm, causes DNA damage, and results in male infertility. Although heat-induced DNA damage in sperm is found across taxa, the mechanisms that prevent heat-induced DNA damage in oocytes are largely unknown. Recent data indicates that heat-stress decreases the repressive chromatin mark H3K9 methylation in the germline of adult C. elegans and has sexually dimorphic localization patterns along DNA in C. elegans spermatocytes and oocytes. H3K9 methylation is established by three primary histone methyltransferases: SET-32, SET-25, and MET-2. In the absence of these histone methyltransferases, there is evidence of increased DNA damage and reduced male fertility. Using a C. elegans mutant strain that lacks all three H3K9 methyltransferases, we are currently assessing the role of H3K9 methylation in heat-induced DNA damage formation during C. elegans spermatogenesis and oogenesis. To visualize and quantify the amount of damage during spermatogenesis, we are using immunofluorescent staining of RAD-51, which marks sites of DNA double strand breaks, in both spermatogenesis and oogenesis. Overall, our ongoing research is developing a better understanding of the role of H3K9 methylation in protecting developing sperm from heat-induced DNA damage and male infertility.

243B Optical manipulation of insulin-like signaling in *Caenorhabditis elegans*

Jim R Mullin, Andrew Gordus CMDB, Johns Hopkins University

Insulin and insulin-like growth factor (IGF) signaling help connect critical functions like growth, reproduction, and aging to an individual's nutritional status. Increased binding of IGFs with the IGF-1 receptor has been observed in human melanomas and carcinomas, whereas in mice, IGF-1 reduction due to dietary restriction can lead to cancer resistance and enhanced lifespan. Similarly, in *Caenorhabditis elegans*, impaired IGF signaling can effectively double an animal's lifespan, though if reduced IGF signaling occurs too early in development, it can result in growth retardation, germline shrinkage, and reduced brood size. These findings suggest that regulation of fluctuations in IGF signaling is critical to controlling aging- and disease-related phenotypes, but currently available transgenic tools do not allow precise and simultaneous control of the spatial and temporal aspects of IGF signaling. This has made it difficult to understand the temporal characterization of IGF signaling dynamics and its influence on animal health.

C. elegans encodes one IGF receptor, the tyrosine receptor kinase DAF-2, that regulates reproductive development, stress resistance, and longevity through the DAF-16/FOXO transcription factor. DAF-2 also regulates the decision to enter dauer stage, an alternative larval stage that promotes survival in unfavorable conditions such as starvation. Previous studies have shown that DAF-2/DAF-16 signaling is required for dauer entry decision, but it remains unclear when and in which cell types signaling is specifically required. To further examine this, I developed a *C. elegans* line expressing optogenetically controlled DAF-2 (Opto-DAF-2) to analyze the dauer decision phenotype after different times and locations of Opto-DAF-2 activation. I plan to examine the spatiotemporal landscape of DAF-2 associated with the dauer entry and exit decision and other phenotypic outcomes.

244B Mechanotransduction in the *C. elegans* digestive tract requires the intermediate filament protein IFA-4

HoJin Nam[1], Rinho Kim[2], Kyuhyung Kim[1][1]Brain Sciences, DGIST, [2]Totipotency, Max Planck Institute of Biochemistry

Cells and tissues are constantly exposed to mechanical forces, which are sensed and integrated by the cytoskeleton to regulate diverse physiological processes. Among cytoskeletal components, intermediate filaments play a crucial role in maintaining cellular integrity and have been implicated in mechanotransduction. However, the mechanisms by which internal structures sense and respond to mechanical forces, particularly the role of intermediate filaments in this process, remain poorly understood. The nematode *C. elegans* has a simple digestive tract consisting of three connected tubes; the pharynx, the pharyngeal-intestinal valve (PI valve), and the intestine. Previously, our lab showed that the PI valve detects intestinal distension and elicits the pharyngeal plunge to mediate food deglutition (Park et al., 2024). However, the underlying mechanism remains largely unknown, particularly how mechanic forces are translated into internal signaling pathways. To further investigate the molecular basis of mechanotransduction in the PI valve, we screened 23 genes known to be expressed in the PI valve and found that deletion mutants of *ifa-4,* an intermediate filament protein gene, exhibit excessive accumulation of foods in the anterior intestinal lumen and also showed defects in the pharyngeal plunge. We then examined the expression pattern of the *ifa-4* gene and found that *ifa-4* is indeed expressed in the PI valve. Moreover, the *ifa-4* mutant phenotypes were fully rescued by the expression of *ifa-4* cDNA under the control of either its own or the PI valve-specific promoter. Furthermore, Ca2+ activity in the PI valve was abolished in *ifa-4* mutants, while optogenetic activation of the PI valve induced pharyngeal plunges in *ifa-4* mutants, similar to control animals, suggesting that *ifa-4* is required for PI valve activation. To further investigate the role of *ifa-4* in mechanotransduction within the PI valve, we are currently performing Translating ribosome affinity purification (TRAP) single-cell RNA sequencing (scRNA-seq) to profile the transcription landscape in the PI valve and identifying *ifa-4*-dependant gene regulatory networks in the PI valve. This study will lead to a further understanding of the molecular mechanisms by which intermediate filaments contribute to mechanotransduction.

245B Visualizing the trafficking route of the polycystins LOV-1 and PKD-2 to cilia and extracellular vesicles in health and disease

Carlos Nava Cruz, Jonathan D Walsh, Elizabeth Desranleau, Inna A Nikonorova, Juan Wang, Maureen M Barr HGINJ, Rutgers- The State University of New Jersey

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in PKD1 and PKD2, encoding polycystin-1 and polycystin-2, respectively. Polycystin-1 and polycystin-2 form a receptor TRP channel complex that localizes to cilia and extracellular vesicles (EVs). EVs are nano-communication devices shed by cells for intercellular signaling. EVs play a role in modulating tissue homeostasis and may contribute to cystogenesis in ADPKD. In *C. elegans*, the polycystins LOV-1 and PKD-2 are required for male mating behavior in male ciliated sensory neurons. LOV-1 and PKD-2 are transported from the endoplasmic reticulum (ER) in the soma, along the dendrites to cilia, where they are shed into the environment as ciliary EVs. We are using *C. elegans* to understand mechanisms regulating polycystin trafficking and the effect of pathogenic mutations on polycystin localization and function.

To model human PKD2 pathogenic mutation polycystin-2C331S, we used CRISPR/Cas9-mediated genome editing to generate the orthologous mutation PKD-2C180S in *C. elegans*. We found that PKD-2C180S resulted in reduced abundance of PKD-2 in the soma to 15% of the wild-type and abolished cilia and ciliary EV localization of PKD-2.

We next analyzed how PKD-2C180S affects the interaction of PKD-2 with LOV-1. PKD-2 is required for LOV-1 cilia and EV localization; in the *pkd-2(sy606)* mutant, LOV-1 remains in the soma and does not localize to cilia or ciliary EVs (Walsh 2022, Nikonorova 2024). Similar to *pkd-2* null mutant, the PKD-2 C180S mutation abolished LOV-1 ciliary and ciliary EV localization, but also reduced levels of LOV-1 in the soma. These data suggest that the PKD-2 C180S mutation is functionally equivalent to the *pkd-2* null allele. We are determining how the PKD-2C180S mutation affects polycystin function by analyzing polycystin mediated male mating behaviors.

We conclude that *C. elegans* is a robust system to study ADPKD pathogenic mutations and disease-associated variants of uncertain significance. Understanding polycystin-1 and polycystin-2 trafficking mechanisms may provide insight into potential therapeutic targets to attenuate or prevent aberrant cilia and EV signaling.

References

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246B Dissecting the contribution of giant KASH proteins to dendritic arborization

Alex Neupauer, Daniel Elnatan, Matthew Laurence, Daniel A Starr, G.W. Gant Luxton UC Davis

Giant KASH proteins are massive (800 kDa to 1 MDa) spectrin repeat-containing outer nuclear membrane proteins that extend into the cytoplasm and play crucial roles in cellular architecture and function. These proteins are implicated in neurodegenerative diseases, including amyotrophic lateral sclerosis and ataxia, yet the pathogenic mechanisms remain unclear. Our research reveals a novel role for ANC-1, the *C. elegans* giant KASH protein ortholog, in maintaining dendritic complexity independent of its canonical nuclear anchoring function. Using high-resolution confocal microscopy, we have identified specific morphological defects in PVD neurons – the only *C. elegans* neuron with elaborate branching patterns – lacking ANC-1. These neurons display three distinct phenotypes: reduced secondary branch formation (42% decrease compared to wild-type), irregular tertiary branch formation with increased tortuosity (1.8-fold higher than controls), and accelerated age-dependent deterioration of the dendritic arbor. Notably, these defects emerge before detectable nuclear positioning abnormalities, suggesting ANC-1 directly regulates dendritic morphogenesis through cytoskeletal organization rather than nuclear anchoring. To quantify these complex morphological changes with enhanced precision, we developed an innovative dual neural network approach. Our first network segments PVD neurons labeled with *ser-2Prom3*::mCherry with exceptional accuracy (intersection over union score of 0.692, well above the 0.5 threshold for successful segmentation), while the second extracts quantitative phenotypes from these segmentations. This approach allows us to analyze potentially hundreds of worms and measure subtle changes in branch density, angle distributions, and temporal patterns of degeneration that would be impossible to quantify manually. By comparing neurons across different ages, we aim to precisely characterize how *anc-1* mutations accelerate age-dependent neurodegeneration and identify crucial time windows for potential therapeutic intervention. This study bridges fundamental cell biology with neurodegenerative disease mechanisms by revealing how nuclear envelope proteins influence neuronal stability and maintenance during aging. Our findings will provide insights into how giant KASH protein dysfunction contributes to neurodegeneration and may identify new therapeutic targets for these debilitating disorders.

247B Novel infection by *Mucor hiemalis* kills *Caenorhabditis* hosts through intestinal perforation

Jay Ni, Jessica Sowa Biology, West Chester University of Pennsylvania

The nematode *Caenorhabditis elegans* is a eukaryotic multicellular organism that has emerged as a popular model system to study cell biology and host-pathogen interactions. Presently, *C. elegans* is studied as a natural host of intracellular pathogens *N. parisii* and Orsay Virus along with other extracellular bacterial and fungal pathogens. *C. elegans* research is limited by the number of naturally occurring pathogens to the organism. Through a sampling project to identify new pathogens of *C. elegans*, we identified the fungus *Mucor hiemalis* as a potential pathogen of *Caenorhabditis* species. We observed the fungus in the intestinal lumen of wild-caught *Caenorhabditis briggsae* and co-culturing the wild-caught species with intracellular pathogen response reporter *C. elegans* confirmed the potential infection by *M. hiemalis*.

This study characterizes the fungal infection of *M. hiemalis* in *Caenorhabditis* nematodes. We investigated food preference of *C. elegans* to investigate if nematodes show preference toward fungal spores or laboratory bacterium OP50. Fluorescence microscopy with fungal staining revealed the life cycle of *M. hiemalis* within multiple *Caenorhabditis* species at varying growth stages. We observed the killing of nematodes by *M. hiemalis* and determined its host range through a series of lifespan assays. Lastly, we observed for common *C. elegans* immunity transcriptional responses and found that *M. hiemalis* does not induce the Intracellular Pathogen Response and other pathogen specific responses seen with previously studied bacterial and fungal pathogens. Characterization of this fungus in Caenorhabditis nematodes will provide new insights into the biology of pathogenic fungi and their hosts' immune systems.

248B Retrograde communication from peripheral tissues in modulating Neuronal Proteostasis and health

Khairun Nisaa, Avinash Sharma, Carlos Giovanni Silva García MCB, Brown University

Aging is a gradual decline of all cellular processes, resulting in deteriorating physiology, metabolism, and cognitive function. With age, disrupted protein homeostasis leads to multiple chronic diseases. Loss of neuronal proteostasis is causally linked to neurodegenerative diseases, including Alzheimer's disease and neuromuscular disorders, such as Amyotrophic Lateral Sclerosis (ALS). Traditionally, research in neuronal proteostasis has focused on nervous system's physiology. However, the molecular mechanisms behind bidirectional inter-tissue communication and whether proteostasis in peripheral tissues regulate proteostasis in neurons is unexplored. Here we used muscle mutants to study how non-neuronal tissues enhance neuronal health.

This study aims to uncover molecular pathways that facilitate communication from peripheral tissues to neurons, focusing on identifying therapeutic targets to delay neurodegeneration. Specifically, we aim to identify muscle-derived markers that mediate the prognosis of neurodegenerative diseases and if they serve as early indicators of neuronal health and degeneration. This will enhance our understanding of the systemic contributions of muscle to aging and examine how neurodegeneration is influenced by altered proteostasis in peripheral tissues.

Therefore, the innovation of this work is that it focuses on understanding the contribution of peripheral tissues that can elicit adaptive responses in neurons.

249B Analysis of *rad-51* separation of function allele suggests divergence of the SDSA and dHJ pathways prior to RAD-51 filament disassembly

Joseph H. Oberlitner[1], Maggie Tinman[1], Aasthika Das[1], Emily Koury[1], Nicola Silva[2], Sarit Smolikove[11]Department of Biology, University of Iowa, [2]Department of Biology, Masaryk University

DNA double-strand breaks (DSBs) are formed during meiosis, so their repair in the homologous recombination (HR) pathway will lead to crossover formation, which is essential for proper chromosome segregation. HR contains two sub pathways: double Holliday junction (dHJ) that generates crossovers, and synthesis dependent strand annealing (SDSA) that creates non-crossovers. RAD-51, an essential protein for the formation of all products of HR, assembles on the processed DSB, allowing the invasion of the ssDNA into an unbroken region of homology. After successful invasion, RAD-51 is removed by RAD-54.L to initiate repair. Here we investigate a separation of function allele of *rad-51*, *rad-51::FLAG,* as well as two other RAD-51 alleles: wild type-like *rad-51::degron* and impaired *GFP::rad-51*. *rad-51::FLAG* displays slowed repair kinetics, resulting in an accumulation of RAD-51 foci*. rad-51::FLAG* worms also exhibit DSB checkpoint activation, but to a less extant than that of *rad-51* null mutants. In a proximity ligation assay, RAD-54.L and RAD-51 show enriched colocalization in *rad-51::FLAG* germlines (but not in *rad-51::degron*), consistent with stalling during the strand invasion step in HR. The defects in RAD-51 disassembly in *rad-51::FLAG* mutants lead to formation of chromosomal fragments, similar in their magnitude to ones observed in *rad-51* or *rad-54.L* null mutants. However, *rad-51::FLAG* mutants, (unlike a *rad-51* null, *GFP::rad-51* or *rad-54.L* null mutants), displayed no defects in the formation of crossover designated sites (via GFP::COSA-1 localization). Given that *rad-51::-FLAG* worms show checkpoint activation and chromosomal fragments, these results suggest that crossover repair concludes normally, while the non-crossover pathway is perturbed. This is strikingly different from *rad-51::degron* and *GFP::rad-51* strains*,* which are proficient or deficient in both pathways, respectively. These results suggest that non-crossovers vs crossovers have distinct recombination intermediates and diverge prior to RAD-51 disassembly.

250B Investigating the molecular mechanism of reproductive dysfunction caused by transgenerational ethanol exposure

Iván Olaya[1], Lisa Truong[2], Patrick Allard[11]Institute for Society and Genetics, University of California, Los Angeles, [2]Human Genetics Graduate Program, University of California, Los Angeles

Meiosis is a specialized cellular program required to produce haploid gametes through two rounds of chromosome segregation (meiosis I and meiosis II). During meiosis I, homologous chromosome segregation is facilitated by recombination and synapsis, which is characterized by the formation of a proteinaceous structure called the synaptonemal complex. Errors in recombination and synapsis can activate meiotic checkpoints that can result in germline apoptosis. Recombination and synapsis can be altered by deregulation of histone acetylation levels. Additionally, acetylation levels are modulated by ethanol, which is metabolized into acetate to provide the acetyl groups for histone acetylation, thereby highlighting the link between the environment, epigenetics and biological processes. Preliminary data in our lab indicates that parental (P0) exposure to ethanol in *C. elegans* causes increased germline apoptosis and embryonic lethality in F3 worms, indicating that ethanol has transgenerational effects on reproduction. However, it is unknown whether the increased apoptosis in F3 germlines is due activation of the recombination and/or synapsis checkpoints in response to meiotic defects. To investigate ethanol's transgenerational effects on reproductive dysfunction, we will monitor the progression of recombination and synapsis in F3 germlines after P0 ethanol exposure by immunofluorescence and high-resolution microscopy to analyze defects in these processes. Furthermore, we will determine the mechanism of F3 germline apoptosis by inactivating the DNA damage checkpoint through a *spo-11* mutant and the synapsis checkpoint via a *pch-2* mutant. Our work will improve our mechanistic understanding of how environmental exposures can have transgenerational effects on reproductive health and how environmental information is inherited.

251B CCPP-1 plays cilia-dependent and cilia-independent roles in fertility

Matt Tsodikov, Jessica Damanski, Jessica Dominguez, Ileana Gonzalez, Olivia Hiltke, Sarah Jardim, isabella Jacobs, Jack Centrelli, Genis Espinal, Nina Peel Biology, TCNJ

Glutamylation, the covalent attachment of glutamic acid to tubulin in the polymerized microtubule, is enriched on long-lived microtubules. It is thought that glutamylation contributes to centriole stability, cilia motility and axon function. Glutamylation of the microtubules is catalyzed by a family of tubulin tyrosine ligase like (TTLL) enzymes; deglutamylation is catalyzed by CCPP-1. In *C. elegans,* mutation of ccpp-1 is associated with hyperglutamylation and ciliary degeneration. We have found that *ccpp-1* mutations also lead to a reduction in brood size which is not rescued by mating with WT males. Concomitant loss of glutamylating enzymes rescues the brood size defect indicating that the phenotype is due to hyperglutamylation. Using the *che-10(e1809)* mutant we find that cilia loss does not fully recapitulate the *ccpp-1* brood size defect suggesting that the brood size reduction is only partially due to loss of cilia. In support of this, an *osm-5p::ccpp-1* transgene which drives expression of CCPP-1 in ciliated neurons fully rescues the *ccpp-1* Dyf phenotype, but only partially rescues brood size. Together our data indicate that CCPP-1 contributes to brood size maintenance via both cilia-dependent and cilia-independent functions.

252C Spatial distribution of PLK-1 regulated by a nuclear envelope protein controls mitotic timing in *C. elegans* embryos

Victória Puccini de Castro[1], Razvan Azamfirei[1], Frances E. Moore[1], Pablo Lara-Gonzalez[2], Shirin Bahmanyar[1]1Molecular, Cellular and Developmental Biology, Yale University, [2]University of California Irvine

Nuclear Envelope Transmembrane Protein (NET)31 was first identified through proteomics screens for nuclear envelope (NE) enriched proteins in mouse cells. Studies in trypanosomes, plants and human cells suggest that NET31 interacts with components of the Nuclear Pore Complex (NPC); however, its specific function at the NPC remains unclear. Here, we used *C. elegans* embryos to investigate the function of the nematode homologue of NET31, CeNET31. Quantitative high resolution fluorescence imaging of the first mitotic division revealed that CeNET31 is involved in multiple mitotic events. Its loss accelerates chromosome condensation kinetics, mislocalizes mitotic chromosomes to the nuclear periphery, and slows lamina disassembly. We hypothesized that the function of CeNET31 may be related to the other NPC components (NPP-1, NPP-4 and NPP-11) that promote NE disassembly by serving as docking sites for Polo-Like Kinase (PLK)-1. Consistent with this, CeNET31 depletion, like NPP-1 depletion, resulted in a specific loss of PLK-1 recruitment at the NE. However, uniquely upon CeNET31 loss, PLK-1 was enriched in the nucleoplasm, suggesting a distinct role for CeNET31 in regulating the spatial distribution of PLK-1. This redistribution of PLK-1 upon loss of CeNET31 may explain the mitotic defects observed at both the NE and within the nucleus. A key function of nuclear PLK-1 is to ensure timely mitosis through recruitment of CDC-20, the activator of the anaphase promoting complex, to kinetochores. Consistent with an increased pool of nuclear PLK-1 in CeNET31-depleted embryos, CDC-20 was recruited to kinetochores earlier, correlating with faster progression through mitosis, which was exacerbated by partial depletion of Cyclin B1. Thus, we identify CeNET31 as a key regulator of mitotic timing in embryos through its role in spatial regulation of PLK-1.

253C An extragenic enhancer of the *pix-1* phenotype in muscle is *ipmk-1*

Hiroshi Qadota[1], Robert Hudson[1], Yuchu (Richard) Wang[2], April Reedy[2], Jasmine C. Moody[2], Guy M Benian[2]1Pathology, Emory University, [2]Emory University

In *C. elegans* body wall muscle, integrin adhesion complexes (IACs) are found at the bases of M-lines and dense bodies, and at muscle cell boundaries (MCBs). We reported that screening of the Million Mutation Project (MMP) by immunostaining with antibodies to PAT-6 (a-parvin), an IAC component, led to discovery that the PIX-1 pathway is required for assembly of IACs at MCBs (Moody et al., 2020). PIX-1 is a RacGEF that activates CED-10 (Rac), and *pix-1* mutants have less activated (GTP bound) CED-10 in muscle. We also reported that the GAP for the PIX-1 pathway in muscle is RRC-1 (Moody et al. 2024). We suspected that the original MMP strain, VC20386, contains an enhancer of the Pix-1 phenotype because after outcrossing VC20386 to wild type and recovering *pix-1 (gk299374)*, VC20386 shows both lack of accumulation of PAT-6 at the MCB, and a large gap between muscle cells whereas the outcrossed *pix-1* strain derived from it, shows only lack of accumulation of PAT-6 at the MCB. By crossing VC20386 to *pix-1(gk299374)* 5X OC strain, in the F2 generation ¼ showed the enhanced phenotype suggesting that enhancement is due to a recessive mutation in a single gene. After 2 more crosses and checking whether genes at the ends of each chromosome were wild type or had the VC20386 mutations, we mapped the enhancer to the left end of IV, where there are mutations in 11 genes. Based on SAGE, 4 /11 genes are expressed in muscle, and we made double mutants of each of these four with *pix-1*. One gene, *ipmk-1*, when combined with *pix-1*, yielded the enhanced phenotype. IPMK-1 is an inositol phosphate multikinase and converts IP3 to IP4, IP4 to IP5, and PIP2 to PIP3. By itself, *ipmk-1(tm2687)* shows dramatic large accumulations of PAT-6 at MCBs. Yang et al. (2021) reported that i*pmk-1(tm2687)* has retarded post-embryonic growth and a prolonged defecation cycle, and that this phenotype can be rescued by mutating *ipp-5*, which encodes inositol 5-phosphatase, which converts IP3 to IP2. We are currently determining if other IACs components are mis-localized in *ipmk-1*, conducting transgenic rescue, determining whether double mutants with other members of the PIX pathway show enhancement, developing an antibody to IPMK-1 to localize it, and determining if an *ipp-5* mutant can rescue our *ipmk-1* MCB defect. Our working hypothesis is that localized formation of PIP3 by IPMK-1 is crucial for localization of the IACs including the PIX-1 complex to specific locations near the muscle cell membrane.

254C HUM-7, a type IX unconventional myosin, is a novel regulator of integrin adhesion complexes in *C. elegans* muscle

Hiroshi Qadota[1], Stefano Derossi[2], Yuchu (Richard) Wang[2], Jasmine Moody[2], Guy M Benian[2]1Emory Univ, [2]Emory University

In vertebrate striated muscle, much of the force of muscle contraction is transmitted to the outside of the cell via “costameres”, which are muscle-specific “integrin adhesion complexes” (IACs; aka focal adhesions). Costameres attach the myofibrils located at the perimeter of the muscle cell to the muscle cell membrane and overlying extracellular matrix and occur at each sarcomeric Z-disk. In the striated muscle of *C. elegans*, IACs reside at 3 locations—the bases of the sarcomeric M-lines and dense bodies (Z-disks) and at the muscle cell boundaries (MCBs). Each IAC consists of the heterodimeric transmembrane protein integrin and many proteins associated with it both intra- and extracellularly. The MCBs contain only a subset of proteins found at dense bodies. In a screen for mutants with defects in the MCB, we identified the gene *pix-1*, which encodes a RacGEF (guanine nucleotide exchange factor) (Moody et al., 2020) and the gene *rrc-1*, which encodes a RacGAP (GTPase activating protein) (Moody et al., 2024). During the RacGAP screening, we also found that *hum-7* mutants have a defect at the MCB. The HUM-7 protein is predicted to have the following domains: An RA (Ras association) domain, a myosin head domain, 4 consecutive IQ domains, 2 C1 (phorbol ester/diacylglycerol binding) domains, and a RhoGAP domain. Based on the sequence of its myosin motor domain and the presence of the other domains, HUM-7 is a class IX unconventional myosin. Two deletion mutants of *hum-7* show less accumulation of PAT-6 (alpha-parvin) at MCBs, similar to *pix-1* and *rrc-1* mutants. Since the HUM-7 contains myosin head and RhoGAP domains, we next examined which domain is crucial for HUM-7’s function at MCBs. We investigated missense mutations in the myosin head and RhoGAP domains. Two of three missense mutations in the myosin head region show the MCB defect, but two missense mutations in the RhoGAP domain, including mutation of an amino acid required for RhoGAP function (*pj63*), did not show the defect. We prepared an antibody against HUM-7, and localized HUM-7 in muscle cells. By confocal microscopy, anti-HUM-7 antibodies localize between dense bodies and at the MCB but flanking the location of IAC components in body wall muscle cells. This is a fascinating localization pattern and contrasts with the location of PIX-1 and RRC-1 which localize to the M-lines, dense bodies and the MCB. Our working hypothesis is that HUM-7 works as a “truck” to deliver IAC components to the MCB region.

255C Investigating the role of a conserved 14-3-3 protein, FTT-2, in smooth muscle contractility

Mustafi Raisa Amin Biology, Northeastern University

Proper regulation of cell contractility is crucial for the regulation of several biological systems, including respiratory, digestive, and cardiovascular systems. In the hermaphroditic nematode *C. elegans*, the reproductive system includes a contractile tube of myoepithelial cells called the spermatheca. This structure stores sperm and serves as the site for oocyte fertilization. Controlled contraction of the spermatheca facilitates the embryo's movement into the uterus. Cell contractility in the spermatheca relies on actin and myosin. It is also, in part, controlled by Ca2+ signaling through the phospholipase PLC-1, which facilitates Ca2+ release from the endoplasmic reticulum. Previous research has demonstrated the involvement of the heterotrimeric G-protein alpha subunit, GSA-1/Gαs, and protein kinase KIN-1/PKA-C in ovulation and Ca2+ release. However, the upstream regulators of these pathways remain unidentified. The spermatheca is not under neuronal control. So, how do the spermathecal cells know when to contract? To identify novel regulators of spermathecal contractility, we performed a candidate RNAi screen to look for genes that caused an increase in the number of occupied spermathecae. One of the candidate genes, *ftt-2*, is one of the two putative 14-3-3 encoding genes in *C. elegans*. The 14-3-3 proteins have been implicated in a growing number of cell biology processes; however, little is known about their involvement in the regulation of cellular contraction. Hence, the study of the FTT-2 gene may identify a novel function of the 14-3-3 protein.

256C A model for non-muscle myosin based contractility in endosomal tubule fission

Wilmer Rodriguez, Anne Norris, Barth Grant Molecular Biology and Biochemistry, Rutgers University

After endocytosis, plasma membrane components enter the endocytic system, destined for degradation in the lysosome or recycling back to the plasma membrane. In many cell types, early and recycling endosomes form a dynamic network of vesicles and tubules, particularly in the C. elegans intestine. Fusion and fission within this network are essential for cargo transport during endocytic recycling. We previously showed that SDPN-1/Syndapin is necessary for basolateral endocytic recycling in the C. elegans intestine, interacting with the PXF-1 protein, a Rap-GTPase exchange factor. Mutations that block SDPN-1/PXF-1 binding, as well as loss of RAP-1, disrupt recycling. Rap-GTPases can negatively regulate RhoA activity, prompting us to test if SDPN-1 regulates RHO-1/RhoA on endosomes. Consistent with a negative regulatory relationship, we found that depletion of RHO-1 suppressed sdpn-1 mutant recycling defects, indicating that control of RHO-1 activity is a key mechanism by which SDPN-1 promotes endocytic recycling. RHO-1/RhoA is well-known for controlling actomyosin contraction cycles. Little is known about non-muscle myosin II (NMII) contractility on endosomes, but membrane tension is key for many membrane fission events. Our analysis found that loss of SDPN-1 or RAP-1 elevates RHO-1(GTP) levels on intestinal endosomes, and depletion of NMII-isoform NMY-1 also suppressed sdpn-1 mutant recycling defects, suggesting that NMY-1 is a key target for RHO-1 regulation during recycling. Importantly, we found that SDPN-1 and PXF-1 proteins are largely restricted to the vesicular elements of the tubulovesicular network, while other components also label the tubules. NMY-1 and NMY-2 are present on or near these compartments. We propose that endosomal tubule fission from vesicular compartments requires local control of membrane tension mediated by SDPN-1-based down-regulation of RHO-1/actomyosin-based tension at the junction. This could be accompanied by maintained tension along the tubule length and recruitment of fission/constriction factors such as Dynamin or RME-1 at the junction. Consistent with this proposal, we found that 80% of tubule fission events in the intestinal endosomal network occur at vesicle-tubule junctions. Further dynamics analysis and identification of additional components in this pathway are underway.

257C Exploring Glycolysis in the Primary Cilium of *Caenorhabditis* elegans

Victoria Nuñez Barón[1], Gustavo Salinas[2], Laura Romanelli Cedrez[11]Institut Pasteur de Montevideo, [2]Institut Pasteur de Montevideo-Universidad de la República

ATP production primarily relies on glycolysis and oxidative phosphorylation, with the latter being the most energy-efficient pathway. However, in tissues with high energy demands and rapid fluctuations in consumption, the Warburg effect occurs, favoring glycolysis even in the presence of oxygen. In the nervous system, synaptic energy requirements are addressed not only by mitochondrial enrichment at synapses but also by the formation of a 'glycolytic metabolon' -a transient assembly of glycolytic enzymes that enhances pathway efficiency and accelerates ATP production.

The primary cilium, a cell-surface organelle, plays a crucial role in mediating cellular communication with its environment. The formation, maintenance, and sensory functions of this organelle are likely to require substantial energy. While mitochondria at the ciliary base have been identified as the primary ATP source, it remains unclear whether ATP diffusion from the base to the apical end is sufficient to supply all energetic demands. Given that aerobic glycolysis serves as an alternative energy source in high-demand conditions, such as in the axons of *C. elegans* motor neurons, we question whether a similar mechanism operates within the cilium.

The present work explores whether glycolysis occurs in the primary cilium and, if so, whether a glycolytic metabolon is constitutively present or induced under conditions of high energy demand or oxygen deprivation. Specifically, we aim to determine the subcellular localization of the glycolytic enzymes phosphofructokinase (PFK-1.1) and aldolase (ALDO-1), as well as assess morphological phenotypes in a set of ciliated neurons from mutant strains for these genes using the DiI dye. Our results indicate no abnormalities in DiI incorporation, suggesting that ciliary structure remains unaffected in the absence of these functional enzyme activity.

We are currently generating transgenic organisms expressing either the glycolytic enzyme PFK-1.1 or ALDO-1 fused to the mScarlet reporter under a ciliated neuron-specific promoter, each co-expressed with a ciliary protein fused to GFP as an organelle marker. Preliminary results indicate that PFK-1.1 colocalizes with the ciliary marker at the periciliary membrane compartment.

258C TBC-2, a RAB-5 GAP antagonizes Insulin signaling through endosomal regulation of CNK-1

Soumyendu Saha, Christian Rocheleau Anatomy and Cell Biology, McGill University

Insulin/IGF signaling (IIS) in *C. elegans* regulates stress responses and life span. DAF-2/ Insulin-like Growth Factor Receptor activates the AGE-1 PI3 kinase to generate PI(3,4,5)P3 which recruits downstream kinases PDK-1 and AKT-1. PDK-1 phosphorylates and activates AKT-1 which in turn phosphorylates the DAF-16 transcription factor to sequester it away from the nucleus. Under stress conditions, insulin signaling is downregulated and this results in a loss of FOXO phosphorylation resulting in nuclear localization. While we understand the genetic components of the insulin signaling pathway, the spatial regulation of these components remains less understood. We have previously reported that loss of TBC-2, a RAB-5 GTPase activating protein involved in endosome maturation, results in increased localization of DAF-16 to endosomes at the expense of nuclear localization. This had downstream effects in reduced longevity and downregulated DAF-16 controlled gene expression.

We found that AKT-1, but not upstream Insulin signaling components localized to DAF-16 positive endosomes in the *C. elegans* intestine suggesting that TBC-2 functions at the level of AKT-1. We found that TBC-2 physically interacts with CNK-1 (Connector Enhancer of KSR-1), which is involved in Insulin signaling in mammals and RAS signaling in both mammals and *C. elegans*. Loss of function of CNK-1 supressed endosomal localization of AKT-1, indicating the novel role of CNK-1 in IIS regulation in *C. elegans*. In *tbc-2* mutants, CNK-1 localizes to DAF-16 positive endosomes in a manner that is partially dependent on DAF-2, PI3K and PDK-1, but not AKT-1. Knockdown of CNK-1 showed suppression of the endosomal localized DAF-16. CNK-1 acts as a positive regulator of IIS by regulating DAF-16 localization and conferring higher lipid accumulation and heat stress tolerance. CNK-1 also enhances expression of the DAF-16 target gene, *sod-3*. We hypothesize that TBC-2 antagonises the IIS by binding and regulating the free population of CNK-1. In *tbc-2* mutants, CNK-1 promotes AKT-1 activation on endosomes to inhibit DAF-16.

259C Chromosomal rearrangements alter the crossover distribution

Akihide Onishi, Neo Ogawa, Takamune T Saito Genetic Engineering, Kindai University

Crossover formation between homologous chromosomes is essential for proper chromosome segregation during meiosis I. In *C. elegans*, the distribution of individual crossovers is primarily concentrated in the arm regions of the chromosome. In contrast, their formation in the central region of the chromosome is repressed. Chromosome rearrangements such as heterozygous inversions and translocations have been recognized as crossover suppressors. This property has been used strategically to facilitate the maintenance of recessive lethal mutations and to mainten the linkage of multiple mutations on the same chromosome, as a balancer chromosome. In this study, we measured global crossover formation during oogenesis in *C. elegans* heterozygous for *mIn1*, a 9.15 Mb (~60% of total length) inversion at the central region of chromosome II. To quantify the crossover formation in Bristol/Hawaiian hybrid, progenies after mating with Bristol males were corrected to each PCR tube. We determined the origin of each chromosomal site by SNP genotyping using the TaqMan assay with real-time PCR. We used the four single nucleotide polymorphism (SNP) sites located at the two ends of a chromosome and at the boundaries between the arms and the central region as markers. By aligning the results of the four SNP markers, we determined and quantified where the crossover formed on the left arm, middle, or right arm of each chromosome. In the full-length chromosome II, the crossover frequency was 49.6 cM for the wild-type control and 49.4 cM for the *+/mIn1* inversion heterozygote. This crossover frequency indicates that single crossover per homologous chromosome pair in both cases. The wild-type control showed a higher crossover frequency on the arms, 23.9 cM on the left arm and 19.8 cM on the right arm, while the crossover frequency in the central region of the chromosome II was reduced to 5.8 cM. In the *mIn1* inversion heterozygotes, as expected no crossover was observed in the central region. In contrast to the control, the crossover frequency was extremely shifted to the left arm, where the pairing center is located, 47.6 cM in the left arm and 2.4 cM in the right arm in *+/mIn1*. Further investigation is need to determin whether the bias in the distribution of crossovers in the heterozygous inversion in the central region is generally affected by pairing centers. In flies, heterozygous inversions have been shown to increase the frequency of crossovers in normal pairs of homologous chromosomes, a phenomenon referred to as the interchromosomal effect. We examined in the *C. elegans +/mIn1* strain whether heterozygous inversion increases crossover formation in normal pairs of homologous chromosomes and found that the crossover frequency in chromosome V was comparable to that in the wild-type control. This finding suggests that the heterozygous inversion itself does not contribute to the interchromosomal effect in *C. elegans*.

260C Genetic suppressor screen of separase mutants identifies cohesin subunits

Cody Saraceno[1], Michael Melesse[2], Dillon Sloan[3], Amy Fabritius[4], Harold Smith[5], Andy Golden[5], Györgyi Csankovszki[6], Joshua Bembenek[71]Obstetrics and Gynecology, Wayne State University, [2]Oak Ridge National Laboratory, [3]University of North Carolina at Chapel Hill, [4]UC Davis, [5]NIH, [6]University of Michigan, [7]Wayne State University

Separase is a well-conserved protease best known for its function in promoting anaphase onset by cleaving cohesin. In the *C. elegans* oocyte, it was demonstrated that separase has a role in the formation of the eggshell by promoting cortical granule exocytosis just after the onset of meiosis I anaphase. Notably, this is a role independent of chromosome segregation which still requires its proteolytic activity. To elucidate the mechanism of separase activity during cortical granule exocytosis, we conducted an ENU mutagenesis screen for suppressors of a temperature sensitive, partial separation-of-function allele of separase that covered nearly a million haploid *C. elegans* genomes. At the restrictive temperature, this allele has minimal issues in chromosome segregation but fails to localize to or exocytose cortical granules. In our screen, we identified 68 suppressor mutations of this allele in 7 different genes, including 14 intragenic suppressors, 47 mutations in *pph-5*, and 7 mutations in previously unidentified genes including *hsp-90* (which regulates *pph-5*), and 3 cohesin genes not directly cleaved by separase. Interestingly, while the mutations in these cohesin genes suppress the lethality associated with this allele, RNAi depletion at varying levels does not. This suggests that the mechanism of suppression is not simply due to a loss of cohesin function. Our future plans are aimed at verifying these suppressors by CRISPR and investigating the mechanism of suppression by observing the cellular phenotypes of these cohesin mutants. It will also be interesting to see if these cohesin alleles can restore separase localization to vesicles and if they can rescue eggshell defects caused by the incomplete exocytosis of vesicles.

261C Intermediate filaments shape host-microbe interactions in C. *elegans*

Jadirah Sarmad, Philipp Kolodziej, Fatemeh Maniei, Florian Geisler Institute of Molecular and Cellular Anatomy, RWTH Aachen University

Intermediate filaments (IFs) are essential cytoskeletal components that maintain intestinal epithelial integrity, a key defence against microbial invasion. However, their precise role in host-microbe interactions remains unclear. Using *Caenorhabditis elegans* as a genetically tractable model, we examined two IF mutants—one lacking an IF network (*ifb-2(kc14)*) and another with an altered IF network (*ifd-2(bz187)*)—and compared them to a control strain with an intact IF network. Worms were exposed to the CeMbio kit microbes, with *E. coli* OP50 serving as a microbial control.

Our findings indicate that the absence or impairment of the IF network can significantly impact lifespan, microbial colonisation, innate immune response, and IF protein expression, suggesting that IFs play a crucial role in regulating microbial interactions, potentially by influencing gut barrier function. Currently, we are investigating how these microbes affect gut morphology in our IF mutants, including gut permeability assays and extended incubation periods to assess the long-term effects of microbial exposure.

Ultimately, this work will help elucidate the molecular pathways by which IFs contribute to gut integrity, providing insights that could inform strategies for maintaining gastrointestinal health in more complex organisms.

262C The CYK-4 GAP domain regulates the cortical targeting of centralspindlin to promote positive feedback during contractile ring assembly and facilitates ring dissolution

Aleesa Schlientz[1], Kian-Yong Lee[1], Sebastian Gomez-Cavazos[1], Pablo Lara-Gonzalez[1,2], Arshad Desai[1], Karen Oegema[11]University of California, San Diego, [2]University of California, Irvine

During cytokinesis, an equatorial contractile ring partitions the cell contents. Contractile ring assembly requires active GTP-bound RhoA generated by the guanine nucleotide exchange factor ECT-2. ECT-2 is activated by centralspindlin, a complex composed of two molecules each of ZEN-4/kinesin-6 and CYK-4. Centralspindlin is activated at the central spindle, which forms between the separating chromosomes during anaphase, and diffuses to the plasma membrane where it activates ECT-2. The N-terminal half of CYK-4 activates ECT-2, whereas the C-terminal half contains a lipid-binding C1 domain that contributes to plasma membrane targeting and a GTPase-activating protein (GAP) domain that has an interaction surface for a Rho family GTPase. The functions of the CYK-4 GAP domain remain unclear. Using the one-cell stage *C. elegans* embryo, we show that RhoA and the Rho binding interface of the CYK-4 GAP domain drive recruitment centralspindlin to the equatorial cortex. A catalytic mutant that disrupts the ability of the GAP domain to convert RhoA-GTP to RhoA-GDP, but does not prevent RhoA binding, exhibited a strikingly different phenotype in which the dissipation of centralspindlin from the cortex at the end of cytokinesis was substantially delayed. Our data suggest that positive feedback in which centralspindlin recruitment drives the generation of active RhoA, and active RhoA drives centralspindlin recruitment, is central to the mechanism that initiates contractile ring assembly. They also indicate that conversion of RhoA-GTP to RhoA-GDP by the GAP domain contributes to the release of centralspindlin from the cortex to ensure timely dissolution of the contractile ring.

263C Defining the mechanism of action of the chromosomal passenger complex during cytokinesis

Aleesa Schlientz, Sebastian Gomez-Cavazos, Arshad Desai, Karen Oegema University of California, San Diego

The chromosomal passenger complex (CPC), whose active subunit is the kinase Aurora B, localizes to chromosomes during mitosis and to the central spindle following anaphase onset. The CPC is essential for cytokinesis, but its mechanism of action remains to be defined. Here, we use the *C. elegans* embryo to analyze the relationship between the CPC and centralspindlin, a heterotetramer composed of dimers of ZEN-4/kinesin-6 and the RhoGAP CYK-4. Centralspindlin diffuses from the central spindle to the cortex, where it activates the RhoGEF ECT-2 to generate active RhoA, the main driver of contractile ring assembly. Inhibition of either the CPC or centralspindlin results in essentially identical cytokinesis defects. Using an assay that monitors centralspindlin localization, we found that the CPC is required to recruit centralspindlin to the equatorial cortex. Prior work suggested that centralspindlin is inhibited by binding of the 14-3-3 protein PAR-5 to a phosphorylated site (S682) on ZEN-4. Phosphorylation of an adjacent site (S680) by the CPC is proposed to activate centralspindlin by dissociating PAR-5. This model predicts that preventing S680 phosphorylation should mimic centralspindlin inhibition. Contrary to this expectation, we found that mutating S680 to alanine had no effect on cytokinesis or embryonic viability. Thus, the CPC target site(s) required for cytokinesis remain unknown. To determine if the CPC targets another site in centralspindlin, we took an unbiased approach, mutating candidate Aurora B sites in CYK-4 and ZEN-4 to alanines in regional clusters. From analysis of 8 cluster mutants representing 34 total candidate sites, only one cluster mutant exhibited cytokinesis failure and penetrant embryonic lethality. This mutant consists of 5 S/T residues located on the surface of the CYK4 GAP domain that faces its lipid-binding C1 domain and in a loop connecting the GAP and C1 domains. Our prior work showed that mutation of even one C1 or GAP domain in the CYK4 dimer prevents the cortical recruitment of centralspindlin. Thus, we speculate that phosphorylation of this region by the CPC facilitates the engagement of the C1 and GAP domains in centralspindlin with the cortex. We are currently defining the critical sites to test if phosphorylation by the CPC alters the conformation of centralspindlin, potentially alleviating an autoinhibitory conformation, to enable its cortical recruitment.

264C Uncovering the role of the CELF/Bruno protein ETR-1 in germline apoptosis

Anna Allen, Farzaneh Shahabi Biology, Howard University

The ELAV-Type RNA-binding protein ETR-1 is one of two CELF/Bruno-family RNA-binding proteins that exist in *C. elegans*. The ETR-1 pre-mRNA is highly alternatively spliced generating at least 19 reported coding transcripts and 1 non-coding transcript, suggesting that ETR-1 functions in an isoform-specific and spatio-temporal manner. Intriguingly, exons 8 and 10 are mutually exclusive and never appear in the same isoform. ETR-1 is developmentally essential, with genetic null animals being embryonic lethal. Previous mutational and RNAi studies have demonstrated that ETR-1 is required for multiple developmental processes including muscle development, nervous system development, and reproduction. Our lab has shown that RNAi depletion of ETR-1 results in an accumulation of germline apoptotic corpses in hermaphrodites and a decrease in the animal's overall fertility. Intriguingly, some of the apoptotic corpses are fully engulfed, while others appear to exhibit a failure or delay in engulfment. To elucidate ETR-1's role in engulfment of apoptotic cells, our preliminary data reveals that RNAi co-depletion of ETR-1 and CED-1 engulfment pathway members suppress the *etr-1(RNAi)* reduced fertility phenotype, while members of the CED-5/CED-2/CED-12 engulfment pathway do not. To determine if *etr-1* is partially redundant with existing engulfment genes, we are conducting non-allelic non-complementation tests through construction of double heterozygous strains of known apoptosis engulfment mutant alleles and various *etr-1* alleles [*etr-1(lq61)* or *etr-1(lq133)*]. Both these *etr-1* alleles are mutations in the alternatively-spliced exon 8, thereby only affect a subset of *etr-1* transcripts, and are viable but exhibit between a 60-70% reduction in fertility when compared to wild-type animals. We are examining the aforementioned double heterozygous strains both for their effect on fertility and numbers of germline apoptotic corpses. Ultimately these studies will help us determine if ETR-1 acts in a previously established engulfment pathway or is in a novel pathway. We anticipate these experiments will give us valuable insights into the role of ETR-1 in germline apoptosis.

265C Comparison of SEL-12 and HOP-1 presenilin protein distribution in *C. elegans* embryos

Bryan Shi, Ben Birkenfeld, Caroline Goutte Biochemistry & Biophysics, Amherst College

The final step in the Notch signaling pathway is the intramembrane cleavage of the Notch receptor by γ secretase protease. γ secretase consists of an evolutionarily conserved catalytic presenilin subunit and three conserved accessory subunits, APH-1, APH-2/Nicastrin, and PEN-2. In *C. elegans*, as well as in humans, there are two alternative presenilin proteins. In the early *C. elegans* embryo, the two presenilins, *sel-12* and *hop-1*, are redundant for supplying the essential catalytic core of γ secretase. In some compromised genetic backgrounds, the contributions of *sel-12* and *hop-1* seem to differ dramatically, yet it is unknown what molecular or cellular differences these two presenilin versions impart on the efficiency of Notch signaling. As one approach to understanding the differences between SEL-12 and HOP-1 functionality, we have used CRISPR-Cas9 to introduce epitope tags to the endogenous genes. Here we present a direct comparison of SEL-12 and HOP-1 subcellular distribution in 4-cell embryos where Notch signaling and responding cells are well defined. By confocal microscopy analysis of immunostained embryos, we show that both presenilins are localized to the plasma membrane and internal membranes of all four blastomeres, and we compare this localization to that of the GLP-1 Notch receptor. The similarity in SEL-12 and HOP-1 protein levels and distribution is consistent with their redundant ability to carry out Notch activation in a wild-type context. We then consider possible differences in assembly and trafficking of SEL-12 versus HOP-1 in compromised genetic backgrounds where one of the other three subunits is unavailable. SEL-12 and HOP-1 show a similar dependence on the APH-1 and APH-2 subunits for localization to the plasma membrane, and we are currently comparing the effect of PEN-2 removal. Thus far, our results are most consistent with a model in which the two alternative presenilin proteins are present at equivalent levels in the 4-cell embryo, and participate in γ secretase assembly and trafficking in a similar way, leaving enzymatic efficiency as a possible explanation for any differences in functionality between SEL-12 versus HOP-1.

266C Regulation of MSP polymerization by a CK1 kinase and an intrinsically disordered protein

Nirvanjyoti Sharma Shimul[1], Sophia De La Vega Mireles[2], Boston T Andrew[1], J. Matthew Ragle[2], Jordan D Ward[2], Diane C Shakes[11]Biology, William & Mary, [2]Molecular, Cell, & Developmental Biology, UC Santa Cruz

Many specialized cells, like sperm cells, employ unique strategies to regulate their cytoskeleton. During sperm development, the spermatocytes of *Caenorhabditis elegans* cease transcription and translation and discard the well-known cytoskeletal proteins actin and tubulin by segregating them into residual bodies. As a result, the mature sperm locomote not via actin or tubulin but via the Major Sperm Protein (MSP) – a distinct cytoskeletal protein that dynamically assembles and disassembles within the pseudopod to drive sperm crawling. Within spermatocytes, MSP is sequestered in bundled polymer structures called fibrous bodies (FBs) which assemble on the cytosolic side of Golgi-derived membranous organelles (MOs). In our current model of FB assembly, the CK1 kinase SPE-6 is required for MSP polymerization whereas the intrinsically disordered protein SPE-18 ensures that MSP polymerizes and bundles at the cytosolic face of the MO. In a quest to identify other factors that might regulate FB formation, we generated knockout mutants in C09B9.4, a gene that is both highly expressed in sperm and is the closest homolog to SPE-6. Knockout hermaphrodites exhibit sperm-specific fertility defects; they produce very few viable progeny but large numbers of unfertilized oocytes. Notably, C09B9.4 knockout spermatocytes fail to assemble MSP into FBs. In addition, C09B9.4 is the right place to regulate FB formation as CO9B9.4::3XFLAG localizes to the FBs. Like SPE-18, C09B9.4 begins to be expressed in mid-pachytene spermatocytes, segregates to spermatids during the budding division, but then disappears quickly thereafter. Our current studies suggest that the absence of C09B9.4 disrupts the normal patterns of multiple FB localized proteins including SPE-18. However, unlike *spe-6* null spermatocytes which arrest undivided and in prometaphase I, C09B9.4 KO spermatocytes undergo meiotic divisions and form spermatids although much of the cell's MSP is aberrantly lost in the residual bodies during post-meiotic budding stage. C09B9.4 KO sperm exhibit impaired sperm activation, and any pseudopods that form are abnormally small. In ongoing studies, we are investigating how C09B9.4 kinase functions together with SPE-6, SPE-18, and other accessory proteins to promote proper FB assembly.

267C Characterization of telomere binding proteins in *Caenorhabditis elegans*

Jan Sluka[1], Alexandra Blake[1], Nadezda Podvalnaya[2], Emily Nischwitz[3], Sabrina Dietz[2], Albert Fradera-Sola[1,2], Lars Teschke[1], Rene Ketting[2], Falk Butter[11]Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, [2]Institute of Molecular Biology, [3]Diagnostic and interventional radiology, Universitaets Klinikum Heidelberg

Telomeres are located at the ends of chromosomes bound by specialized proteins that protect them from DNA damage repair and control the homeostasis of telomere length. In *C. elegans*, our current knowledge of the proteins that interact with the telomeric sequence is incomplete. We previously used quantitative interactomics to identify proteins interacting with telomeric repeats. Here, we characterize DVE-1, one of these candidates. While DVE-1 displays a lethal knockout phenotype and is involved in chromatin remodeling, it has not previously been studied in the context of telomeres.

We demonstrate that recombinantly expressed DVE-1 binds with nanomolar affinity to single-stranded telomeric DNA in a microscale thermophoresis assay independently of other proteins. In telomere pull-down experiments, recombinantly expressed fragments of DVE-1 revealed its specific telomere binding region and domain. Using RNA interference, we performed a *dve-1* knockdown and showed the effects on gene regulation by RNA sequencing as well as the phenotypic consequences. Finally, using quantitative mass spectrometry data, we show a possible interaction of DVE-1 with the nucleosome and remodeling (NuRD) complex. We hypothesize that DVE-1 acts at telomeres as a recruitment support for the NuRD complex and thus has a homologous function to the ZNF827 protein in humans to regulate and maintain telomere length.

268C Strategies toward the identification of telomerase RNA component in the nematode

Hobum Song[1], Seonhong Kim[2], Hee-Jung Choi[2], Junho Lee[21]Institute of Molecular Biology and Genetics, Seoul National University, [2]Seoul National University

Telomeres are chromosomal end structures crucial for preserving genome integrity in eukaryotes. The regulation of telomere length primarily depends on telomerase, a ribonucleoprotein complex with a catalytic core comprising telomerase reverse transcriptase (TRT-1/TERT) and telomerase RNA component (TERC). Despite extensive studies of telomerase across various model organisms, the TERC remains unidentified in the nematode. While the identification of TERC is complicated by the lack of its conserved features, the limited expression and activity of telomerase in the nematode further challenge the process. To address this gap in knowledge, we have developed two complementary approaches using C. elegans as a model system: 1) an in vitro method using recombinant C. elegans TRT-1 to pull down its specific RNA binding partner from total C. elegans RNA, and 2) an in vivo approach utilizing germline proliferation mutant strains to obtain sufficient telomerase for immunoprecipitation techniques (e.g. RIP, CLIP). Candidates of TERC revealed by analyzing the RNA sequencing data will be screened by verifying the presence of a template region containing approximately 1.5 repeats of the telomeric repeat motif – the only conserved region in TERC across diverse organisms. Subsequently, genetic methods and telomerase repeated amplification protocol (TRAP) will validate the true C. elegans TERC. The successful identification of C. elegans TERC would significantly advance our understanding of both nematode biology and telomere biology beyond C. elegans. Furthermore, this research may establish a pipeline that promises to facilitate TERC identification in less-studied organisms and provides broader insights into protein-nucleic acid interactions research.

269C Exploring tissue-specific mechanisms and requirements for Nucleolin organization

Lio A Cook[1], Noah A Lind[1], Hugo Schodet[1,2], Jesutomisin Olajuyin[1], Eleanor Wuichet[1,3], Muthumeena Ramanathan[1,4], Catherine S Sharp[1], Dustin L Updike[1], Emily L Spaulding[11]MDI Biological Laboratory, [2]Paul Sabatier University, [3]Davidson College, [4]University of Maine

Precise organization of cellular space is essential for control over complex biological reactions. For example, the RNA binding protein, Nucleolin (NCL), displays precise organization within the nucleolus. This organization is considered critical for ribosome biogenesis and is disrupted in cancer and neurodegeneration. Because NCL is an essential gene in vertebrate animal models and no clear homolog exists in *Drosophila*, most studies of NCL organization use *in vitro* models. However, the nucleolus is exquisitely sensitive to environment and *in vitro* conditions can influence nucleolar dynamics. As a result, mechanisms that regulate NCL organization are unclear. We discovered the *C. elegans* homolog of NCL, named NUCL-1. Deleting endogenous NUCL-1 results in viable worms, providing the opportunity to explore mechanisms of NCL organization in a living animal. We recently used CRISPR/Cas9 genetic engineering to develop a split-fluor system to visualize NUCL-1 in germ cell nucleoli. Combining this system with super-resolution (AiryScan) imaging, we mapped the intricate sub-nucleolar organization of germ cells in living *C. elegans*. Precise sub-nucleolar organization is thought to be essential for ribosome biogenesis, but our *in vivo* work challenges this canonical view. Full deletion of the *nucl-1* gene causes impaired fertility, delayed development, extended lifespan, and smaller body size, all phenotypes linked to impaired ribosome biogenesis. In contrast, deleting the arginine-glycine (RG) repeat domain from endogenous NUCL-1 disrupts sub-nucleolar organization, but worms are healthy and fertile with motor hyperactivity. These results decouple precise sub-nucleolar organization from phenotypes associated with ribosome biogenesis and lead to the hypothesis that there are tissue-specific requirements for sub-nucleolar organization. We are expanding the split-fluor system to extend our study of NUCL-1 organization to all major somatic tissues, starting with neurons. Using this system, we are assessing sub-nucleolar organization across neuronal subtypes and determining how the NUCL-1 RG repeat domain contributes to that organization. We are also performing a battery of phenotypic tests linked to the function of individual neuron types using the NUCL-1 strains. Uncovering tissue-specific patterns, mechanisms, and functions of sub-nucleolar organization will lay the groundwork for understanding the role of NCL organization in human disease.

270C Deciphering Synaptonemal Complex Protein Networks: A Cross-Species Perspective

Autumn P Stronach, Lisa N Petrella Biological Sciences, Marquette University

The Synaptonemal Complex (SC) is a protein structure integral to meiosis that fails to assemble correctly under temperature stress, resulting in asynapsis and loss of fertility. The evolutionary signature of the SC central element SYP proteins is unique. SYP proteins have strongly conserved secondary and quaternary structure, but extremely divergent primary amino acid sequence even between closely related species, maintaining only 24-50% identity between orthologs. In *C. elegans* there are six SYP proteins, SYP-1, SYP-2, SYP-3, SYP-4, and the SYP-5/6 paralogs. Each of these central element proteins is interdependent on each other for formation of the central element, as such mutants in any result in asynapsis, failure to form crossovers, and sterility. In *C. elegans,* interactions of these SYP proteins to form the final structure of the central element have been mapped through various techniques including yeast-two hybrid. While there are homologs present for all of the SYP proteins across the *Caenorhabditis* phyla, it is unknown if the interaction patterns between the SYP proteins within the central element are conserved. We are interested in understanding more about the protein network making up the central element of the SC in related species of *Caenorhabditis*. This project investigates two aims related to this gap in knowledge; firstly, we aim to determine if this pattern of protein interactions during SC assembly is conserved between closely related *Caenorhabditis* species, *C. briggsae* and *C. tropicalis*. Secondly, should these interactions prove to be conserved across species, we aim to determine if cross-species SYP protein interaction is feasible due the conserved secondary structure of the proteins. In this study we employ a yeast two hybrid model to test interactions between *C. elegans* SYP proteins and SYP proteins of similar species *C. briggsae* and *C. tropicalis*. This study will allow us to better understand the methods of adaption employed by different *Caenorhabditis* nematodes to maintain proper meiotic progression, opening avenues for future work in understanding the evolutionary mechanisms of fertility.

271C An *ex ovo* culture protocol for *Caenorhabditis elegans* embryos

Clover Stubbert, Cherry Soe, Pavak Shah University of California Los Angeles

The impermeability of the C. elegans eggshell constrains experimental approaches to manipulating and labeling embryonic cells, especially at later developmental stages. We have found that a minimal media based on the Bianchi Lab's protocols for culturing C. elegans neurons for electrophysiology is able to support embryo viability and development through "hatching" after removal of the eggshell via enzymatic digestion. These embryos retain a membrane that constrains the embryo during elongation and twitching and >90% survive to L1 with >90% surviving to adulthood while producing progeny after recovery on NGM plates. Most losses appear to be due to mechanical damage likely occurring during the pipetting and transfer of the delicate embryos after eggshell removal. We have tested a wide range of toxins, dyes and inhibitors including taxol, Janelia Fluor, colchicine, ciliobrevin B, dynarrestin, and jasplakinolide among others, and found all to be effective at concentrations significantly lower than reported for mammalian cell culture and in perm-1 RNAi embryos. Ex ovo culture of worm embryos will thus be a useful tool for cell and developmental biology, allowing the fluorescent imaging capabilities of the worm to be complemented by the large catalog of small molecules available.

272C Assembly and Clearance of the Pre-cuticle matrix

Sage Aviles[1], Chelsea Darwin[1], Prioty Sarwar[1], Helen Schmidt[2], Nicholas Serra[2], Meera Sundaram[11]Genetics, University of Pennsylvania School of Medicine, [2]University of Pennsylvania School of Medicine

C. elegans must synthesize and shed its external cuticle multiple times throughout its life cycle. During this molt cycle, there is a transient and molecularly distinct «pre-cuticle» apical extracellular matrix (aECM) that precedes the collagenous cuticle and helps to pattern its proper structure. This pre-cuticle is composed of zona pellucida (ZP) domain proteins, lipocalins, Hedgehog-related (Hh-r) proteins, and various other glycoproteins, many of which are similar to those found in/near mammalian aECMs. Its specific composition varies between tissues and stages and therefore is one key readout of cell fate. During embryonic and larval morphogenesis, the pre-cuticle lines and helps shape epithelial surfaces, including the interior lumens of tubes. The pre-cuticle also helps shape cuticle ridges, the alae, that decorate the adult surface. Finally, some pre-cuticle proteins are required for molting or for the barrier function and overall integrity of the cuticle.

Precuticle protein localization is quite dynamic. The proteins are first apically secreted in an unpatterned way, then assembled into discrete layers or substructures that remain for only a couple of hours, before being disassembled and re-endocytosed. The factors that control these various steps are largely unknown. We will report on several factors that promote pre-cuticle assembly or endocytosis

273C Dying cells are recognized by transthyretin-like TTR-53 for phagocytosis

Dylan Suriadinata[1], Chinmay Phadke[2], Ann M Wehman[11]Biochemistry & Biophysics, Texas A&M University, [2]Texas A&M University

Phagocytic cargos expose lipids to signal for engulfment, which is important for tissue homeostasis and immune responses. The transthyretin-like protein TTR-52 recognizes phosphatidylserine (PtdSer) for receptor-mediated phagocytosis but is not required for the engulfment of all dying cells in *Caenorhabditis elegans*. Therefore, we asked whether closely related proteins, TTR-53 and TTR-54, were required for phagocytosis. We discovered that TTR-53 promotes phagocytosis of dying polar bodies, while *ttr-54* deletion had no effect on their internalization timing. StayGold-tagged TTR-53 localizes to the surface of phagocytic cargos, including dying polar bodies and large extracellular vesicles such as cytokinetic midbody remnants. Disrupting PtdSer asymmetry using *tat-1* mutants does not change TTR-53::StayGold localization, revealing that TTR-53 is unlikely to interact with PtdSer. In contrast, TTR-53::StayGold relocalized in *tat-5* mutants, suggesting that TTR-53 may instead bind a lipid whose asymmetry is regulated by TAT-5. In preliminary experiments, purified TTR-53 bound to PIP lipids, revealing new potential lipids translocated by TAT-5. These data indicate that dying cell recognition depends on signaling from other lipids in addition to PtdSer. This study also identifies TTR-53 as a novel lipid probe.

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274C The shift in DSB repair pathway choice is executed in mid-pachytene in *C. elegans* meiosis.

Hui Tian[1], Korie Campbell[2], Sarit Smolikove[11]Biology, University of Iowa, [2]University of Iowa

During meiosis, SPO-11 induced DNA double-strand breaks (DSBs) are repaired via two different mechanisms: 1) the double Holliday junction (dHJ) pathway which generates exchange of DNA molecules to establish crossovers (COs); 2) the synthesis-dependent strand annealing (SDSA) pathway produces non-crossovers (NCOs). The mechanism governing the choice between these pathways remains unclear. In *C. elegans* meiosis, both pathways are active, with each nucleus averaging 6 COs (one per each chromosome). The standard model in the field is that each DSB, regardless of when it is created, has the potential to be repaired through the dHJ or SDSA pathway. However, our recent finding (Hicks *et al.* 2022) showed that this decision is made at the time of DSB formation. DSBs that form from meiotic entry to mid-pachytene stage of meiosis ("early DSBs") are distinct from DSBs formed from mid to late pachytene ("late DSBs"). Early DSBs do not form COs and therefore are destined to form NCOs while late DSBs can generate both COs and NCOs. It was clear that the transition between these repair modes happens in mid-pachytene stage of meiosis However, since only a single time point was tested it was unclear if the decision is made at the point of reaching mid-pachytene or, for example, after ~20h beyond meiosis entry has elapsed. First, we quantified germline growth (from 0 to 48 hours post-L4) in our system and found that extension of the germline is mainly contributed by the expansion of the pachytene stage, consistent with other studies. To be able to calculate in which germline region where CO-proficient DSBs are created, we used photoconversion of Dendra2::histone. We used this tool to label nuclei and track their position at later timepoints, to precisely measure of the rate of movement of meiotic nuclei in the germline. We found that nuclei movement gradually slows down as the germline develops, which influences the timing and positioning of CO-proficient DSB formation. Finally, we use SPO-11 auxin mediated degradation, but varied the time in which COs (via COSA-1 foci) were analyzed. We found that more time is required to eliminate COs as the germline grows (from 0 to 48 hours post L4), but this extension is proportional to germline growth. Hence, concurrently with germline growth, the region of pre-CO DSBs expands, but the transition between early and late DSBs remains at the mid-pachytene region, where the peak in the number of recombination intermediates (RAD-51 foci) is found. Thus, it is not the time spent in meiotic prophase, but rather the meiotic stage that determines the transition between the DSB repair modes.

275C Targeted RNAi screens reveal novel regulators of RNA-binding protein phase transitions in *Caenorhabditis elegans* oocytes

Victoria E Tice[1], Mohamed T Elaswad[1], Grace M Thomas[1], Christya Haddad[2], Ashley Cichon[3], Alexis Royer[4], Ethan Harris[2], Leah Thermel[2], Helsa Thomas[2], Corrin Hayes[1], Jennifer Schisa[11]Biology, Central Michigan University, [2]Central Michigan University, [3]Central Michigan university, [4]Central Michigan University

The regulation of maternal mRNAs is essential for oogenesis and the production of viable gametes. Recent findings suggest the tight regulation of RNA-binding protein (RBP) phase transitions contributes not only to mRNA metabolism, but also to the maintenance of oocyte quality. Our lab is exploring the regulation and function of RBP phase transitions during oogenesis. Here, we describe two approaches to identifying inhibitors of ectopic condensation of RBPs in maturing oocytes. In the first targeted RNAi screen, we asked the extent to which the regulatory network of RHO-1 aggregate clearance also modulates phase transitions of RBPs in oocytes. This question stemmed from the common findings of the chaperonin-containing tailless complex polypeptide 1 (CCT) chaperonin, actin, and CGH-1 as regulators of both processes. We screened candidates from seven GO categories as inhibitors of ectopic MEX-3 condensation. We identified six novel regulators, and we established that genes involved in lysosome acidification, regulation of mitochondria membrane potential, and ESCRT-complex-mediated autophagy are not required to inhibit ectopic MEX-3 condensation. Thus, the regulatory pathway of MEX-3 phase transitions is overlapping, but distinct from RHO-1 aggregate clearance during oocyte maturation. Building on this screen's discovery of two genes in the Coat Protein Complex II (COPII complex) and one gene in the COPI complex, we are currently expanding our investigation to address the following questions: 1) To what extent are COPII and COPI complex genes required to inhibit ectopic MEX-3 condensation? 2) Do COPII or COPI complex genes prevent the ectopic condensation of other RNA-binding proteins? 3) Do COPII and/or COPI modulate RBP phase transitions by altering cytoskeletal elements or by preventing the ectopic assembly of ER sheets? 4) Do the alterations in RBP phase transitions caused by depletions of COPII or COPI result in abrogated RBP functions in regulating mRNA? Our results to date suggest subsets of the COPII and COPI complexes are required to inhibit ectopic condensation of MEX-3 and CAR-1. We hope our studies identifying novel regulators of phase transitions during oogenesis will ultimately provide insight into the causes of poor-quality oocytes.

276C LINCing lifespan to cytoplasmic biophysics: Giant KASH proteins regulate age-dependent changes in *C. elegans* cellular architecture

Zoe Upham, GW Gant Luxton, Daniel Starr, Daniel Elnatan University of California Davis

Aging is characterized by progressive deterioration of intracellular mechanisms, including loss of proteostasis and altered cellular communication. These hallmarks are associated with changes in cytoplasmic biophysical properties, yet the causal relationships between cytoplasmic biophysical properties (e.g., constraint and macromolecular crowding) and aging have remained elusive due to technical limitations in measuring these properties in living organisms. Our lab pioneered an innovative approach to quantify cytoplasmic biophysical properties in intact *C. elegans* tissues using genetically-encoded multimeric nanoparticle (GEM)-based passive nanorheology. By tracking the diffusive behavior of 40 nm fluorescent GEMs expressed in the cytoplasm of intestinal cells, we quantified dramatic age-dependent changes in cytoplasmic biophysical properties. In wild-type animals, GEM diffusion decreases 12-fold during aging, from 5.36 x 10-3 µm2 /s in day 1 adults to 4.47 x 10-4 µm2 /s by day 7 ($p<0.0001$), revealing significant changes in cytoplasmic architecture with age. Our research has uncovered distinct regulatory mechanisms governing cytoplasmic biophysical properties: ribosomes primarily control cytoplasmic macromolecular crowding, while the giant KASH protein ANC-1 specifically regulates cytoplasmic constraint. Remarkably, *anc-1* null mutants display a 2.7-fold increase in cytoplasmic GEM diffusion compared to wild-type animals at day 1 (1.43 x 10-2 µm2 /s vs. 5.36 x 10-3 µm2 /s, $p<0.0001$), demonstrating ANC-1's critical role in establishing cytoplasmic constraint in young adults. However, by day 7, GEM diffusion in *anc-1* null mutants converges with wild-type (3.88 x 10-4 µm2/s vs. 4.47 x 10-4 µm2 /s, not significant), suggesting age-dependent compensation or the emergence of alternative constraint mechanisms. The discovery of separate regulatory pathways for cytoplasmic macromolecular crowding vs. constraint provides a novel framework for understanding how cellular architecture changes during aging. Our findings reveal an unexpected role for giant KASH proteins in regulating cytoplasmic biophysical properties, independent of their canonical function in nuclear positioning, and suggest new approaches for investigating aging-associated cellular deterioration.

277C FISH Analysis of Programmed DNA Elimination in *Caenorhabditis auriculariae*

Naoki Uwatoko[1], Nami Haruta[1], Tokio Nakamura[1], Simo Sun[2], Taisei Kikuchi[2], Asako Sugimoto[1]1Graduate School of Life Sciences, Tohoku University, [2]Graduate School of Frontier Sciences, The University of Tokyo

While multicellular organisms generally maintain identical DNA composition across all cells, programmed DNA elimination (PDE), also known as chromatin diminution, represents a striking exception. In PDE, specific DNA segments are selectively discarded in certain cell types. Although this phenomenon was first discovered over a century ago in a parasitic nematode and has since been observed in other species, its molecular mechanisms and biological significance remain largely unknown.

In this study, we investigate PDE in *Caenorhabditis auriculariae*, which diverged early in the *Caenorhabditis* genus. The genome assembly suggested that the *C. auriculariae* germline cells possess 2n=12 chromosomes, whereas somatic cells exhibit 2n=26. This difference arises from the elimination of about 2% (2.4 Mb) of the genome in somatic cells. This DNA loss occurs through cleavage of subtelomeric and internal chromosomal regions, leading to the removal of approximately 300 genes and repetitive DNA sequences. Subsequently, new telomeres were added to the newly formed chromosome ends.

To determine the timing of PDE during development, we performed fluorescence *in situ* hybridization (FISH) using probes targeting telomeric repeats and repetitive sequences from the eliminated regions. We found that PDE occurs in somatic precursor cells between the 8- and 16-cell embryonic stages. Within these cells, telomeric repeats became undetectable during interphase, and after mitosis, the internally cleaved fragments were detected in the cytoplasm. The eliminated chromosome fragments were subsequently degraded after several rounds of cell division.

We also conducted a small-scale RNAi screening for PDE-related genes in *C. auriculariae*, and identified several genes whose knockdown suppressed PDE and resulted in late embryonic or early larval lethality. These finding raise a possibility that PDE is an essential process for the development of *C. auriculariae,* and further studies will elucidate its physiological significance.

278C Inhibitor-2 motif docking onto Protein Phosphatase 1 mediates hand-off to adapters involved in chromosome segregation

Neha Varshney[1], Johnathan Meaders[1], Karen Oegema[1,2], Arshad Desai[1,2]1Department of Cell & Developmental Biology, University of California San Diego, [2]Department of Cellular and Molecular Medicine, University of California San Diego

The catalytic subunit of Protein phosphatase 1 (PP1c) executes a major fraction of Ser/Thr dephosphorylation events. PP1c activity is regulated by a conserved set of 3 accessory proteins: Sds22, Inhibitor-2 and Inhibitor-3. Using a live imaging-based phenotypic screen, we found that depletion of SDS-22 or Inhibitor-3 resulted in a severe phenotype that mimicked loss of PP1 function. By contrast knockdown of Inhibitor-2 (SZY-2; referred to as Inh2) leads to a milder phenotype with delays in chromosome alignment and anaphase onset similar to those caused by disruption of PP1c docking on the kinetochore adaptor protein KNL-1. Both Inh2 and KNL-1 employ SILK and RVxF motifs for PP1c docking and thus cannot stably both be bound to PP1c; I-2 possesses an additional motif (HYNE) that binds to and blocks the PP1c active site. These results suggest that Inh2 functions as a reservoir for holding PP1c prior to its hand off to mitotic adaptors like KNL-1. Thus, while Inh2 is a potent PP1c inhibitor *in vitro*, it functions as a selective positive regulator of PP1c functions *in vivo*. To understand in greater depth how Inh2 promotes specific PP1c functions, we used a transgene-based replacement system to selectively disrupt specific Inh2 interfaces with PP1c. We found that the mutation of the RVxF motif in Inh2 unexpectedly resulted in a more severe phenotype than Inh2 depletion; simultaneous mutation of the HYNE motif that blocks the PP1c active site, suppressed the severe RVxF mutant phenotype. These results suggest that mutation of the Inh2 RVxF motif leads to "dead-end" Inh2-PP1c complexes where the Inh2 HYNE motif blocks PP1c activity and cannot be handed off to adaptors like KNL-1. Overall, these results suggest that Inh2 functions as a dynamic reservoir for holding mature PP1c and facilitating its handoff to mitotic adapters like KNL-1, and that recognition of the RVxF-bound state of the Inh2-PP1c complex is critical for the handoff.

279C Using MAPH-9 to investigate the formation and maintenance of microtubule doublets

Nabor Vazquez Martinez, Jeremy Magescas, Michael Tran, Jessica Feldman Biology, Stanford University

Cilia are microtubule-based organelles important for signaling and/or motility in eukaryotes, and cilia defects are associated with human disorders known as ciliopathies. The cytoskeletal core of each cilium called the axoneme is composed of a radially symmetrical configuration of nine microtubule doublets (MTDs). Unlike single-cylinder microtubules in the cytoplasm, MTDs feature a distinctive configuration formed by building an incomplete microtubule off the wall of an existing microtubule. Despite the conservation of this structure across the tree of life, how MTDs form and are maintained *in vivo* is still poorly understood. Previous technical and biological limitations have hampered our understanding of MTD formation *in vivo*, however, *C. elegans* provide an advantageous system in which to dissect this process. Unlike most other ciliated organisms, *C. elegans* centrioles are composed of nine singlet microtubules during early development but centrioles complete their MTDs just prior to ciliogenesis. We have identified MAPH-9/MAP9 as a microtubule associated protein that exclusively recognizes MTDs in *C. elegans*. MAPH-9 is only expressed in ciliated cells, MAPH-9 localization appears concomitant to the post-mitotic appearance of microtubule doublets, and MAPH-9 localizes to the basal body and outgrowing axoneme. The mammalian MAPH-9 homolog, MAP9, also localizes to the axoneme of mammalian cells and mouse tissue, suggesting that MAPH-9/MAP9 plays a conserved role. Loss of MAPH-9 caused ultrastructural MTD defects and perturbed cilia function. The specificity of MAPH-9 to MTDs enables us to carry out a forward genetic screen and proximity labeling approach to better understand molecules important in MTD formation. We are also investigating how MAPH-9 specifically recognizes MTDs by analyzing the necessity and sufficiency of various domains of MAPH-9 in MTD localization. This study holds significance in advancing our understanding of ciliary structure, shedding light on the fundamental process of MTD formation.

280C Identification of resistance alleles to intracellular infection using natural variation in *Oscheius tipulae*

Candyd Lace R Velasquez[1], Robert J Luallen[1], Marie-Anne Felix[2], Gaotian Zhang[21]Biology, San Diego State University, [2]Institute of Biology of the Ecole Normale Supérieure

Intracellular bacterial pathogens can invade host cells and exploit host machinery for replication. By using genetic tools and manipulation, nematodes serve as a great model for understanding host resistance to intracellular infection. *B. atropi* was discovered in our lab as an intracellular bacterial pathogen that utilizes filamentation to spread from cell to cell in the intestine. To see if there was natural variation to intracellular bacterial infection in *O. tipulae*, we tested a small panel of wild isolates. Different isolates of O. tipulae were found to have a spectrum of susceptibility to *B. atropi* infection, anywhere from a 40-75% infection rate. Interestingly, we found one strain, JU457, that appeared to be resistant with less than a 4% infection rate, while the WT strain CEW1 had a 75% infection rate.

To identify the alleles associated with resistance, we crossed JU457 to CEW1 and conducted bulk segregant analysis (BSA) on the F2 progeny. We used quantitative trait loci mapping (QTL) identify regions associated with resistance. Our mapping found two regions enriched in JU457 alleles, a large region on chromosome 2 of approx.3,000 kb and a smaller region in chromosome 3 of approx. 1,750 kb. We searched for candidate genes on chromosome 3 and found that JU457 has a small insertion in the gene dual oxidase (duox), resulting in a truncated Duox lacking the domain that produces hydrogen peroxide. While Duox is known to produce hydrogen peroxide in response to infection, we hypothesize that *B atropi* might use hydrogen peroxide as a signal for virulence, resulting in a loss of infection in JU457 when the gene is killed. Overall, we have identified genomic regions associated with resistance to intracellular bacterial infection, with a candidate immune factor that may be coopted to facilitate bacterial infection.

281C Organelle crosstalk among mitochondria, cilia, and extracellular vesicles

Juan Wang[1], Carlos Nava Cruz[1], Jonathan Dietz[2], Christopher Rongo[2], Inna A Nikonorova[1], Maureen M Barr[11]Department of Genetics, Rutgers University, [2]Waksman Institute of Microbiology, Rutgers University

Cilia and extracellular vesicles (EVs) are essential for intercellular communication in health and disease. Cilia are microtubule-based, hair-like protrusions that allow cells to survey their environment. Cilia also transmit information in the form of tiny EVs. We aim to understand how EV biogenesis links to signal transmission. How and why cells release EVs are key questions in decoding the cellular language of EVs. We have developed *C. elegans* to study ciliary EVs in vivo. The TRP channel receptor complex proteins LOV-1 and PKD-2 are co-localized in cilia and ciliary EVs in male-specific sensory neurons. The coiled-coil protein CIL-7 is required for ciliary EV biogenesis. Here, we report that LOV-1, but not PKD-2, is associated with mitochondria in the soma, while CIL-7 associates with mitochondria in both soma and dendrites of sensory neurons. The dynamin-related protein DRP-1 is required for mitochondrial fission, and, when mutated, alters mitochondrial morphology and mitochondrial-association of LOV-1 and CIL-7, further demonstrating the mitochondrial localization of these proteins. We are currently testing the hypothesis that mitochondria are key players in regulating ciliary EV biogenesis, despite being physically separated by the ciliary transition zone barrier. Our work opens a new window to understand how mitochondria and cellular metabolism influence ciliary signaling and EV biogenesis.

282C Investigating the role of H3K9 methylation in heat-induced transposon excision

Hannah R Wilson, Daela Montgomery, Nicole Kurhanewicz, Diana E Libuda Biology, University of Oregon

The proper development of haploid gametes, such as sperm and eggs, is critical for sexually reproducing organisms to faithfully pass their genome onto the next generation. Environmental stressors, such as acute heat stress can negatively impact genomic integrity during gamete development, which can lead to genetic mutations and infertility. Although heat-induced male infertility is well-conserved across organisms, the molecular mechanisms behind this sexually dimorphic heat-induced male infertility is relatively unknown. In developing Caenorhabditis elegans sperm, acute heat stress is associated with increased DNA damage, excision and movement of DNA transposons, and male infertility. During gamete production, DNA transposon activity is tightly regulated by conserved mechanisms to maintain genomic integrity including chromatin modifications. The repressive chromatin modification, histone H3 lysine 9 trimethylation (H3K9me3), protects the genome from transposition by regulating the accessibility of heterochromatin thereby repressing the expression of DNA transposons. Using a combination of genetics, cytology, and genomics, we are determining the sexually dimorphic role of H3K9 methylation in heat-induced transposon excision in developing C. elegans germ cells. Using single, double, and triple H3K9 methyltransferase mutants (set-32; met-2 set-25) and immunofluorescence, we find that H3K9 methylation represses heat-induced DNA damage during oogenesis. Additionally, we find that H3K9 methylation differentially regulates heat-induced expression of specific members of the Tc1/mariner transposon superfamily during oogenesis. Our ongoing work indicates that acute heat stress perturbs H3K9me3 in a sexually dimorphic manner in developing germ cells. To determine whether H3K9me3 is associated with heat-induced transposon excision, we are comprehensively defining the heat-induced transposon landscape across the C. elegans genome and comparing this dataset to the sex-specific H3K9me3 landscape. Lastly, we are determining whether the complete loss of histone H3K9 methylation impacts heat-induced male infertility. Overall, these studies will illuminate sexually dimorphic mechanisms that regulate DNA transposon mobilization in developing gametes to maintain genomic integrity for fertility.

283C Nascent Protein Synthesis in Male Meiosis I Drives the MI-MII Transition Through PLK-1-Dependent Centrosome Maturation

Shang-yang Chen[1], Szu-yu Chen[1], Yu-hao Chen[1], Jui-ching Wu[1,2]1Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, [2]Department of Laboratory Medicine, National Taiwan University Hospital

Male meiosis consists of two consecutive divisions, yet whether nascent protein synthesis during meiosis I (MI) is required for meiosis II (MII) remains unclear. Here we demonstrate that translation during MI is essential for the MI-MII transition. Inhibition of nascent protein synthesis in primary spermatocytes allows completion of MI but leads to post-anaphase I arrest, preventing MII entry. While kinetochore structures remain intact, translation-inhibited cells fail to reorganize the division machinery, particularly at the spindle poles. Notably, nascent protein synthesis during MI is required for recruiting Polo-like kinase PLK-1 to centrosomes post-MI, a key step in centrosome maturation and spindle assembly for MII. Inhibiting PLK-1 during MI likewise leads to post-anaphase I arrest. Furthermore, mutation of SPD-5, a pericentrosomal material (PCM) protein, at PLK-1 phosphorylation sites recapitulates the same defect. These findings establish that nascent protein synthesis during MI drives the MI-MII transition by promoting PLK-1-dependent centrosome maturation, ensuring proper male meiotic progression.

284C Twisting Cytokinesis: Cell Adhesion and Cortical Flow Underlie Chiral Morphogenesis in *Caenorhabditis elegans* Embryos

YuXuan (Rain) Xiong, Marcus Lai, Eric Cytrynbaum, Kenji Sugioka University of British Columbia

Chirality is one of the fundamental properties of living systems, wherein the external and internal body structures are asymmetric and cannot be superimposed on their mirror images. In animals, tissue- and organismal-scale chirality arise during morphogenesis, termed chiral morphogenesis. In *Caenorhabditis elegans*, the left-right body asymmetry is specified during the 4-cell stage cell division of the ABa and ABp cells (Wood B., 1991), where twisting motion of the cell cortex, called chiral cortical flow, is observed during cytokinesis. Although chiral cortical flow in worms and snails are proposed to drive chiral morphogenesis (Naganathan *et al*., 2014; Meshcheryakov & Beloussov, 1975), the mechanisms by which cytokinesis establishes embryonic chirality remain elusive. Here, we found that cell adhesion induces chiral cortical flow and cellular rotation in early *C. elegans* embryos. During 4-cell stage cytokinesis, the division axes of ABa and ABp cells are orthogonal to the anterior-posterior axis until early anaphase, then undergo a clockwise tilt, resulting in embryonic chirality. To identify contributors to this division axis tilt, we performed an *in vitro* blastomere isolation assay. We found that attachment of an isolated ABa or ABp cell to a poly-L-lysine-coated glass slide induced chiral cortical flow and clockwise cellular rotation. As previously reported, chiral cortical flow depends on CYK-1/Formin, a conserved actin polymerizing and nucleating factor (Middelkoop *et al*., 2021). Reduction in chiral cortical flow by *cyk-1(RNAi)* decreases cellular rotation, confirming that chiral flow is required for the division axis tilt. However, we also found that cellular rotation requires the attachment of both dividing cell halves to the adhesive surface. These results suggest that 1) adhesion is required for the amplification of intrinsically generated chiral cortical flow, 2) while chiral cortical flow is necessary, it is not sufficient to drive cellular rotation, and 3) a correct adhesion pattern, in addition to chiral cortical flow, is required to induce cellular rotation. Using mathematical simulation, we confirmed that chiral cortical flow and adhesion are sufficient to generate cellular rotation and chiral cellular arrangements. Our study illuminates the novel interplay between cell adhesion and cytokinesis that regulates chiral morphogenesis, which is critical for shaping the embryonic body plan.

285C Exploring endogenous genes associated with exopher increase and probing exopher-associated proteins

Yongzhi Yang, Barth Grant, Monica Driscoll Department of Molecular Biology and Biochemistry, Rutgers University

Exophers are large vesicles, on average 4 µm in diameter, expelled from *C. elegans* neurons, muscle cells, and mammalian cardiomyocytes, kidney cells, and neurons. Baseline exopher production in the mCherry-expressing ALMR neuron is detected between 5-20%, and the numbers usually peak on day 2 of adulthood. While different stress pathways have been shown to increase exopher numbers (including disrupted autophagy, impaired proteasomes, and starvation), endogenous genes that directly associate with exopher increase remain to be determined. To probe this biology, we developed a touch neuron-specific translating ribosome affinity purification (TRAP) assay by expressing the FLAG-tagged RPL-22, a component of the 60S ribosomal subunit, in touch neurons. FLAG antibody allows pull down of ribosomal components and the translating mRNA, enabling touch neuron-specific mRNA sequencing in *C. elegans*. We will compare touch neuron-specific transcriptomes from different adulthood stages, and under different stresses, to figure out key gene expression changes associated with exopher increase. We will examine top candidates using touch neuron-specific RNAi and overexpression to validate their relevance to exopher biology.

Additionally, while we have found that exophers can transport fluorescent proteins and cellular organelles, it is still largely unknown what other cargos and membrane proteins are associated with the exopher. Proximity labeling is a technique that tags the protein of interest with a biotin ligase, such as TurboID, and enables labeling of nearby proteins through biotinylation. We have identified a mutant form of PGL-3, a P granule protein that tends to form a condensate, which is preferentially located in the exopher. Considering PGL-3 as an exopher-enriched cargo, we will express the TurboID-tagged PGL-3 in touch neurons and use mass spectrometry to decode biotinylated proteins inside exopher. We will address the functional relevance of identified proteins using RNAi and protein-tagging.

With the TRAP experiment, we expect to identify gene expression changes that are closely associated with exopher increase; some of these may be crucial for exopher biogenesis. With the proximity labeling method, we anticipate discovering exopher cargoes, cargo receptors, and potentially unique exopher markers. The knowledge gained will advance our understanding of exopher biology and open the door to studying exopher's physiological and pathological roles.

286C Mining Disorder for New Fertility Loci

Yvan Doctorovich[1,2], William McFadden[1,3], Daniel Hochbaum[2], Judith Yanowitz[41]Magee-Womens Research Institute, [2]Universidad Maimonides, [3]Magee-Womens Research Institute/U. Pittsburgh, [4]OBGYN & Reproductive Sciences, Magee-Womens Research Institute/U. Pittsburgh

Intrinsically disordered proteins (IDPs) lack a stable 3D structure but can often adopt structured conformations upon association with binding partners and can induce phase separation to form membrane-less organelles. In the nematode, a number IDPs play key roles in the germ line, including in P granules and the synaptonemal complex. Because these proteins often have poor homology across species, they often appear as orphan proteins with unknown functions. Based on our lab's prior studies with the HIM-5 and GCNA-1 germline-specific proteins that have large disordered regions, we hypothesized that IDPs may be an understudies class of proteins with critical roles in germline biology. We performed global analysis of the 8 nematode proteomes and stratified proteins by percent disorder. We found that across species the proteins with highest degree of disorder tended to be basic suggesting nuclear or ECM functions. We used a transgenerational RNAi feeding screen to assess the contributions of the IDPs to fertility and germ line development. Of the 110 genes tested to date, loss of 15 conferred fertility phenotypes, 10 iof which only presented in the F2 or F3 generation, suggesting they would not have been identified in prior screens.

We have performed detailed analysis of Y40B1B.7, one of the strong sub-fertile loci. AlphaFold suggested this gene is a homolog of human CCDC86 (and therefore, we refer to it as *ccdc-86*) and yeast CGR1, the latter of which has been shown to function in maturation of the 5S ribosome (PMID: 30291245). Complete deletion of the *ccdc-86* leads to slow growth and a delayed switch to oogenesis at adult 3-4 of adulthood, when the first offspring appear. At 25°C, proximal tumors develop in the germ line, similar to *pro-1*, *pro-2*, and *pro-3* and nucleolar mutants, described by the Hubbard lab (PMID: 14973273, 16876152;). CCDC-86::GFP localizes in the nucleolus, surrounding the fibrillar center. *ccdc-86* loss leads to nucleolar fragmentation in many tissues, including the germ line. The interrelationship between CCDC86 and PRO genes in ongoing. We are also addressing functional conservation by asking if human CCDC86 can rescue the null. We have also made a *ccdc-86::AID* to test nucleolar functions in different tissues.

In short, screening the IDP-ome led us to a conserved nucleolar protein that will allow us to interrogate the interrelationship between ribosome maturation and tissue-specific energetic requirements.

287C Conversion of chromosome type from holocentric to monocentric by constructing an artificial kinetochore

Hiroki Yoshida[1], Nami Haruta[1], Tatsuo Fukagawa[2], Tetsuya Hori[2], Asako Sugimoto[11]Graduate School of Life Sciences, Tohoku University, [2]Graduate School of Frontier Biosciences, Osaka University

Many eukaryotes have monocentric chromosomes, where the kinetochore is localized to a specific region of the chromosomes. In contrast, several species possess holocentric chromosomes, in which the kinetochore is distributed along the entire chromosome length. The independent evolution of holocentric chromosomes has occurred in multiple lineages, indicating they confer certain advantages. To better understand the characteristics of these two chromosome types and their evolutions, we attempted to convert the kinetochore position in *Caenorhabditis elegans* from holocentric to monocentric.

To achieve this, we employed an artificial kinetochore system based on the LacO-LacI interaction, which has been previously used in vertebrates (Hori, et.al., J. Cell Biol.2013). This system utilizes the LacO operator and LacI repressor protein from *Escherichia coli*. In our study, part of the *C. elegans* kinetochore protein, HCP-4 (CENP-C ortholog) was replaced with LacI and expressed it as a GFP-fused transgene (GFP::HCP-4::LacI) to target the LacO repeats sequence.

To determine whether the artificial kinetochore was functional in *C. elegans*, we used extrachromosomal arrays containing LacO repeats sequence. In early embryos, GFP::HCP-4::LacI and a control construct, GFP::LacI were observed as single foci in the nucleus, indicating their localization at the LacO array. During mitosis, these GFP foci showed chromosome-like movement and were partitioned into daughter cells. When endogenous HCP-4 was depleted by RNAi, the GFP::HCP-4::LacI foci migrated toward the spindle poles, whereas chromosomes and GFP::LacI were missegregated at the center of the cell. These results suggest that GFP::HCP-4::LacI successfully formed functional artificial kinetochore on the LacO array.

Next, LacO extrachromosomal arrays were integrated into a chromosome to generate an artificial monocentric kinetochore and assessed whether a single chromosome could undergo proper segregation. In the presence of endogenous HCP-4, the GFP::HCP-4::LacI foci localized on the chromosome containing the LacO repeats. Upon depletion of endogenous HCP-4, these GFP::HCP-4::LacI foci separated in a bi-directional manner, accompanied by the chromosomes. This suggested that the artificial kinetochore could facilitate the segregation of a monocentric chromosome in *C. elegans.*

In the future, we aim to integrate LacO repeats into all chromosomes and compare monocentric chromosomes with holocentric chromosomes in *C. elegans*.

288A Homeodomain transcription factors delineate glial diversity

G. Robert Aguilar, Caecilia Thuermer, Ruihuan Yu, Oliver Hobert Department of Biological Sciences, Columbia University

How diverse cell types are generated remains a fundamental problem in developmental biology. The nervous system of *C. elegans* is comprised of the two most diverse cell types in the worm – neurons and glia – and offers a rich setting to study the generation of cell type diversity. Our lab has previously shown that the 118 neuron types of *C. elegans* can be delineated through differential expression of homeodomain transcription factors, which are deployed as terminal selectors of neuron identity. We hypothesize that glial diversity might also be generated by the action of different combinations of homeodomain transcription factors acting as terminal selectors.

Using CRISPR/Cas9-engineered reporter alleles or fosmid-based reporter constructs, we find that the diversity of the 50 ectodermal glia in the worm, classified as either sheath or socket glia, may be described by a code of homeodomain transcription factor expression. Many individual homeodomain proteins are expressed in a “salt-and-pepper” fashion in specific subsets of sheath and/or socket glia, but expression patterns show specific overall themes. For instance, we identify *gfp*-tagged homeodomain transcription factor *pros-1* to be expressed in all sheath glia. Through mutant analysis, we show that *pros-1* controls the terminal features of sheath glia differentiation. An auxin-inducible degron allele of *pros-1* allowed us to demonstrate that *pros-1* is continuously required to maintain sheath glia fate. Taken together, these observations indicate that *pros-1* is a terminal selector of sheath glia fate. Furthermore, deletion of the *pros-1* gene or post-embryonic auxin-induced degradation of PROS-1 protein results in an increase in the number of cells expressing socket markers, suggesting that in addition to promoting sheath glia fate, *pros-1* is continuously required to repress an alternative socket glia fate.

We also identify candidate regulators of pan-glial identity and propose a model to explain the generation of glial diversity in *C. elegans*. Our work advances our understanding of glia developmental genetics in the worm, the genetics underlying cell type diversity, and fills a critical gap in our understanding of glia evolution.

289A Identification of the spatial requirement(s) of the DAF-2 insulin receptor in food type-dependent oogenesis onset and fertilization in *C. elegans*

Asra Akhlaq, Joy Alcedo Department of Biological Sciences, Wayne State University

The nematode worm *Caenorhabditis elegans* is highly genetically tractable and has hundreds of oocytes that can be easily imaged *in vivo*. This makes the worm an excellent model in which to dissect the mechanisms that regulate oocyte biology in response to different diets. Recently, our lab has shown that the *C. elegans* onset of oogenesis and fertilization rates are modulated by the type of bacteria it consumes (1). We have also found that this bacterial-dependent regulation of oogenesis and fertilization require the insulin-like receptor DAF-2 (1). However, the insulin-like peptide (ILP) ligand that regulates onset of oogenesis differs from the ILP(s) that regulate fertilization (1). Because the 40 ILPs in *C. elegans* are expressed in different subsets of cells, this raises the hypothesis that the insulin receptor, DAF-2, acts from different cells to regulate diet-dependent oogenesis onset versus fertilization rates. To test this hypothesis, we are using the auxin-inducible degron system (AID) to determine in which cells DAF-2 is required to modulate oogenesis or fertilization on different bacterial diets. Identification of the cells or tissues in which DAF-2 acts to regulate these two aspects of oocyte biology will potentially delineate two different circuits through which diet regulates oogenesis onset versus fertilization.

Reference:

(1) Mishra et al (2023). eLife 12, e83224

290A Notch pathway regulation of reproductive aging in germline stem cells

Aaron M Anderson[1], Zuzana Kocsisova[1], Tim Schedl[2], Kerry Kornfeld[1]1Department of Developmental Biology, Washington University, [2]Department of Genetics, Washington University

A decline in the number and activity of adult stem cells is a hallmark of mammalian aging. However, mechanisms that regulate the age-related loss of stem cells are poorly defined. Our goal is to leverage the extensive knowledge and experimental power of the *C. elegans* germline stem cell system to understand how adult stem cell activity changes during aging and discover new interventions that can preserve stem cell function and promote healthy aging. Our observational studies identified a rapid, age-related reduction in the number and differentiation of stem cells in the germline of mated hermaphrodites. These changes in stem cell function were strongly correlated with age-related changes in progeny production, suggesting the age-related decline in stem cells is a major contributor to the loss of fertility observed during reproductive aging. Stem cells are maintained in a self-renewal fate through GLP-1/Notch signaling; the GLP-1 receptor promotes transcription of two direct target genes in the stem cells, *lst-1* and *sygl-1*. We observed reductions in the extent of LST-1 and SYGL-1 protein expression in the germline during aging, suggesting that Notch pathway activity declines with age. To elucidate the mechanism of this decline, we evaluated upstream signaling events using smFISH. Similar to SYGL-1 protein expression, the mature cytoplasmic *sygl-1* mRNA levels were significantly reduced in older animals. Surprisingly, active transcription of nascent *sygl-1* mRNA did not display an age-related decline. Our results suggest that the core Notch pathway – ligand, receptor, and activation of transcription – are relatively stable over time. Instead, an age-related change in regulation that functions downstream of transcription, such as mRNA stability, may be the driver of stem cell loss. Consistent with this model, expression of a *sygl-1* transgene with a modified 3' UTR that enhances mRNA stability delayed age-related loss of stem cells and increased mid-life fertility. The CCR4-NOT complex is a conserved RNA degradation system that acts at the 3' end of several transcripts in the germline to mediate proper germ cell development. We depleted a major component of the CCR4-NOT complex in the germline with an auxin inducible system, which resulted in enhanced stability of the *sygl-1* cytoplasmic message. These results indicate that an age-related increase in the activity of the CCR4-NOT complex may reduce the cytoplasmic mRNA levels of Notch target genes during aging. Cumulatively, our work supports a model that post-transcriptional regulation of Notch pathway target genes is an important control point for the age-related decline of germline stem cells and contributes to reproductive aging.

291A Understanding the role of an essential microRNA during early development in *C. elegans*

Orazio Bagno[1], Philipp Dexheimer[2], Luisa Cochella[1]1Molecular Biology and Genetics, Johns Hopkins University, [2]Research Institute of Molecular Pathology

MicroRNAs (miRNAs) are 21-23 nucleotide-long small RNAs that act as post-transcriptional repressors of target messenger RNAs (mRNAs). It is known that production of miRNAs is required for viability in higher metazoans, as disruption of the miRNA biogenesis machinery leads to severe embryonic defects in all model organisms. However, little is known about which miRNAs carry out these essential functions during early development, and what their molecular mechanisms are.

The *miR-35* family (*miR-35fam*) of miRNAs is composed of 8 redundant family members (*miR-35-42*) and essential for *C. elegans* embryogenesis, with loss of the family resulting in completely penetrant embryonic lethality during mid-to-late gastrulation (Alvarez-Saavedra and Horvitz, 2010). *MiR-35fam* is collectively the most highly expressed miRNA in the *C. elegans* germline and embryo, with complete degradation occurring prior to L1. Thus, *miR-35fam* likely does not play a role in post-embryonic development. Despite its importance, the target mRNAs that need to be repressed by *miR-35* to ensure proper embryogenesis remain unknown. Previous work attempting to elucidate the cause of embryonic lethality could not attribute derepression of any individual predicted target of *miR-35fam* to this drastic phenotype (Yang et al., 2020). Additionally, combinatorial derepression of validated targets caused only partially penetrant embryonic lethality. Thus, *miR-35fam* must act by also repressing other targets, which have yet to be elucidated.

To further pinpoint the genes responsible for this phenotype, we performed RNA-sequencing in *miR-35fam* embryos at the 2-cell stage and late gastrulation (shortly before most of these embryos arrest). Strikingly, significant upregulation of the stress-related transcription factor Nrf/SKN-1, along with multiple proteasome, CCT-chaperonin, and heat shock genes was seen at late gastrulation. Moreover, mass spectrometry analysis and mitotracker staining in *miR-35fam* 2-cell embryos revealed an overall increase in protein and mitochondria content, suggesting that *miR-35* may act to control maternal loading and that loss of this control leads to an increased proteostasis response. These observations provide a framework to further understand the function of *miR-35fam* in early *C. elegans* development and perhaps will more generally elucidate the role of miRNA-mediated gene regulation in early metazoan development.

292A Distinct polarity feedback loops regulate the enrichment of the MEX-5 and MEX-6 proteins via PLK-1

Alexandre P. Vaudano, Francoise Schwager, Monica Gotta, Sofia Barbieri University of Geneva

Polarity is a process that segregates proteins at specific localization to enable essential cellular functions like differentiation, cell migration and polarity. At the intracellular level, reaction-diffusion mechanisms sustain the establishment of molecular asymmetries, as observed in the *C. elegans* one-cell embryo where proteins segregate along the antero-posterior axis. Among these, the RNA-binding proteins MEX-5 and MEX-6 enrich at the anterior, leading to the anterior accumulation of the mitotic kinase PLK-1. MEX-5 forms its gradient through differential diffusion, but whether MEX-6 follows a similar mechanism was unknown.

By combining in vivo and in silico approaches, we reveal that MEX-5 and MEX-6 establish gradients through comparable mechanisms, yet they exhibit distinct biophysical properties despite their strong similarity. Our findings suggest that an intricate feedback loop between MEX-5, MEX-6 and PLK-1 regulates their concentration gradients. We genetically dissected this complex circuitry and uncovered that PLK-1 regulates at its turn the anterior recruitment of the MEX proteins, but through two distinct pathways: indirectly for MEX-5, influencing its gradient by regulating cortical polarity, and directly for MEX-6 through physical interaction.

Our study reveals how PLK-1 coordinates polarity and protein gradients through multiple pathways, offering new insights into the molecular logic governing intracellular self-organization. These findings provide a framework for understanding how molecular asymmetries are established and dynamically regulated in polarized cells and how kinase-driven polarity mechanisms orchestrate asymmetric cell division across diverse systems.

293A *spe-58* is a new regulator of sperm development in *Caenorhabditis elegans*

Lyna Benhammou, Noah Dickison, Xue Mei St. John's University

Fertilization is critical to sexual reproduction, requiring the proper development and function of gametes. The nematode *Caenorhabditis elegans* serves as a powerful model for studying fertilization due to its genetic tractability, transparent body, and short life cycle. In *C. elegans*, self-fertilizing hermaphrodites (XX) and males (XO) provide a unique system to study gamete function. This study focuses on characterizing the role of specific genes in fertilization and gamete development. Our focus is on *spe-58*, identified through a mutant allele *as48* that was isolated in a forward mutagenesis screening aiming at discovering genes important for fertilization. The mutant show temperature-sensitive fertility decline, and preliminary analyses suggest that they have poorly differentiated sperm. Sperm activation is a post-meiotic differentiation process by which round spermatids become motile and fertilization competent. Here, *as48* male sperm is assessed using in-vitro activation assays with known activators such as Pronase, Proteinase K, and Zinc. Additionally, *as48* male fertility is evaluated through crossbreeding with feminized *fog-2* mutants, which lack the ability to self-fertilize, ensuring that progeny production depends on male mating. These experiments will provide critical insights into the genetic pathways underlying sperm activation in *C. elegans*, offering broader implications for reproductive biology.

294A Three is a Crowd: Sometimes CED-2 Works with CED-5 and CED-12...Sometimes it Doesn't

Victoria V Brown, Yeshawsi Pulijala Department of Pathology, Rutgers University New Brunswick

The coordinated activation and inhibition of F-actin supports the cell movements of morphogenesis. Among these cellular movements are the engulfment of dying cells during embryogenesis and the larval migration of the distal tip cell. Misregulation of proteins that promote F-actin has been implicated in many diseases, from cancers, to immune diseases, to neuronal disorders. Thus, it is important to investigate the regulation of F-actin. We have shown that C. elegans embryonic epidermal morphogenesis requires the GTPase CED-10/Rac1 to activate branched actin through the WAVE complex. To identify the GEF (Guanine-nucleotide Exchange Factor) that activates CED-10/Rac1 during embryonic cell migrations, we examined the two-component GEF, CED-5/CED-12 (DOCK/ELMO). CED-5/CED-12 is known to activate CED-10/Rac1 to promote both the cellular movements that engulf dying cells during embryogenesis, and a larval distal tip cell (DTC) migration. We tested the involvement of CED-5/CED-12 in embryonic epidermal migrations and found a contradictory result. The loss of CED-5/CED-12 leads to embryonic death due to failed epidermal cell migrations, thus CED-5/CED-12 supports embryonic migrations. However, we found that CED-5/CED-12 inhibited F-actin in the migrating epidermis, the opposite of what was expected for a CED-10/Rac1 GEF. To investigate the two opposing effects of CED-12/CED-5 on F-actin, we investigated if CED-12 harbors GAP (GTPase Activating Protein) functions. A candidate GAP region in CED-12 was mutated using CRISPR, resulting in a CED-12 GAP mutant that has normal corpse engulfment, but failed epidermal migrations due to elevated levels of epidermal F-actin. CED-5 and CED-12 usually collaborate with CED-2/CRKII for all of their previously known functions, including corpse engulfment and gonad migrations. However, when we compared the role in epidermal cell migrations of CED-2 in comparison to CED-5 and CED-12, we found that CED-2 has an opposite role. CED-2 supports F-actin enrichment in the migrating epidermis. This intriguing discrepancy has led us to examine how three proteins (CED-2, CED-5 and CED-12), thought to function together to support the activation of the GTPase CED-10, collaborate to coordinate the essential function of regulating embryonic epidermal F-actin. We will present our findings comparing similar and distinct roles of CED-2 relative to CED-5/CED-12, and likely mechanisms that regulate these actin regulators.

295A Cold shock domain protein LIN-66 coordinates developmental cell fate via microRNA pathway in *C. elegans*

Reyyan F Bulut[1], Victor Ambros[21]UMass Chan Medical School, [2]PMM, UMass Chan Medical School

The core components of the microRNA (miRNA) pathway are well characterized; however, the role of modulatory factors such as microRNA-induced silencing complex (miRISC) cofactors remains less understood. Here, we identify LIN-66, a cold shock domain protein in *Caenorhabditis elegans* previously associated with the heterochronic gene network, as a miRISC cofactor. We find that *lin-66* regulates heterochronic miRNA targets to specify hypodermal seam cell fate while also modulating miRNA-mediated repression of genes involved in neuronal differentiation, embryonic development, and sex determination.

Previously, *lin-66* was linked to *lin-28* regulation via its 3′ UTR. However, our epistasis analysis reveals that *lin-66* functions downstream of the *lin-28/lin-46* axis of the heterochronic pathway. Using endogenous *lin-28* 3′ UTR deletion alleles, we found that *lin-66* does not act through the *lin-28* 3′ UTR or its miRNA target sites. *lin-66* loss-of-function synergizes with *lin-28* gain-of-function alleles, leading to seam cell hyperproliferation. Additionally, *lin-66* mutants ectopically express *LIN-14* in later larval stages, and *lin-14* knockdown rescues the hypodermal seam cell defects of *lin-66(0)* mutants. Our genetic interaction studies demonstrate that lin-66 functionally interacts with core miRISC components. *lin-66* loss-of-function phenotypes are modified by *alg-1* (Argonaute) and *ain-1/ain-2* (GW182 homologs), but not by the *alg-1* functional paralog *alg-2*.

Beyond its role in the heterochronic pathway, *lin-66* also modulates non-heterochronic miRNA activity. We tested *lin-66* depletion in animals compromised for *lsy-6* (ASEL neuronal fate specification) and the *mir-35* family (sex determination). Loss of lin-66 enhances neuronal fate specification defects in *lsy-6* mutants and sex determination defects in *mir-35* family mutants, indicating a broader role in miRNA-mediated gene regulation.

Finally, our bioinformatic and genetic analyses indicate that the cold shock domain of LIN-66 is essential for its function, likely by facilitating miRISC–mRNA interactions. Overall, these findings establish *lin-66* as a pivotal miRISC cofactor, integrating heterochronic and broader miRNA pathways to fine-tune post-transcriptional gene regulation and developmental timing in *C. elegans*.

296A The collagen COL-177 is required for COL-53 localization at specialized sensory aECM

Julia Burnett[1,2], Rachel Swope[1,2], Wendy Fung[1,2], Maxwell G. Heiman[1,21]Department of Genetics, Harvard Medical School, [2]Division of Genetics and Genomics, Boston Children's Hospital

The apical extracellular matrix (aECM) is a network of secreted proteins, lipids, and carbohydrates that lines the external surfaces of epithelia and serves functions ranging from pathogen protection to mechanical integrity. aECM can also exhibit tissue-specific structure and function. For example, sense organs have highly patterned aECM that is specialized for chemosensation, mechanoreception, or vision. A key question is how aECM proteins assemble complex structures in the extracellular environment without molecular motors, cytoskeleton, or even ATP. We discovered that, in *C. elegans* sense organs, glia secrete specialized aECM around the ciliated endings of sensory neurons. We found that cilia of mechanosensory neurons (CEP, OLQ, OLL) are covered by a donut-shaped ring of the glial collagens COL-56 and COL-105, with an inner plug composed of COL-68 and the cuticulin CUTL-17. By contrast, cilia of chemosensory neurons (IL2, CEM, male rays) access the environment by poking through narrow cuticle pores that are also formed by glial-secreted aECM, including the transient protein GRL-18; the collagen COL-53 that lines each pore; as well as COL-68 and CUTL-17. Remarkably, forcing activity of the pore gene expression program in non-pore-forming glia is sufficient to induce formation of an ectopic cuticle pore. To understand how aECM proteins build cuticle pores, we sought to identify factors required for COL-53 localization through a visual forward genetic screen for mutants that mis-localize endogenously tagged COL-53. We isolated two mutants with completely penetrant defects in which COL-53 is expressed in pore-forming glia but fails to localize to pores. These mutants specifically impact COL-53 localization: they do not affect other pore-localized proteins (GRL-18, CUTL-17, COL-68) or grossly disrupt pore formation. Both mutations are X-linked recessive, fail to complement and thus, are likely allelic. Excitingly, the causal mutations lie in COL-177, a collagen that is also expressed in pore-forming glial cells together with COL-53. An essential step in collagen secretion is trimerization in the endoplasmic reticulum. Our current model is that COL-177 trimerizes with COL-53 and is required as a co-factor for its secretion. In summary, our work shows that glial-secreted aECM proteins form specialized structures around sensory cilia, enabling forward genetic screens to identify trans-acting factors that control aECM assembly and patterning.

297A Uncovering mechanisms that govern nucleolar organization and nuclear translation

Wei Cao, Roger Pocock Monash Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash University

Animal reproduction relies on the generation of sperm and oocytes by germline stem cells. Protein synthesis (translation), carried out by ribosomes, is fundamental for the development and function of all living cells. Thus, deciphering ribosome biogenesis regulation is essential for understanding protein synthesis and cellular function. The nucleolus is a membraneless nuclear organelle. In *C. elegans*, the nucleolus is organized into two liquid-liquid phase separated compartments (fibrilla center and granular component) packed with specialised proteins and RNAs to facilitate distinct ribosome biogenesis events. Nucleolar organization differs between cell types and changes in response to cell cycle and stresses. However, much remains unknown about how nucleolar organization is regulated and its functional implications. Furthermore, translation occurs mostly in the cytoplasm, with the mechanism and role of nuclear translation a debated area of research.

In a reverse genetic screen to discover germ cell regulators in *C. elegans*, I discovered that *F23B12.7* is essential for germline development. My subsequent analysis revealed that F23B12.7 regulates nucleolar organization and nuclear translation in *C. elegans* germline stem cells. My unpublished genomic and proteomic analysis found that F23B12.7 and its human homolog CEBPZ (CCAAT/enhancer-binding protein zeta) share DNA binding target genes and protein interactors, suggesting that F23B12.7 function is highly conserved. F23B12.7 homologs have been implicated in ribosome biogenesis, pre-ribosome nuclear export, transcription, and translation, suggesting that regulation of these mechanisms may underpin F23B12.7's role in regulating nucleolar organization and nuclear translation. Deciphering these mechanisms will enable me to uncover fundamental processes governing nucleolar organization and protein synthesis in germline stem cells.

298A Clathrin regulates nucleolar organization - who knew?

Wei Cao, Roger Pocock Monash Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash University

The nucleolus is a membraneless nuclear organelle. In *C. elegans*, the nucleolus is packed with thousands of proteins and RNAs organized into two compartments (fibrilla center and granular component) via liquid-liquid phase separation. The main function of the nucleolus is ribosome biogenesis, which is fundamental for protein synthesis. The nucleolus also has important roles in cell cycle progression, cellular senescence, RNA editing, protein quality control, and viral infection. Nucleolar organization differs between cell types and changes in response to cell cycle and stresses. However, much remains unknown about how nucleolar organization is regulated and its functional implications. I recently discovered that clathrin adaptors, APG-1 and APM-1, play an essential role in nucleolar organization in germline stem cells.

Clathrin and clathrin adaptors mediate endocytosis and intracellular transport but have no known nuclear function. I used RNAi to knockdown clathrin and the remaining clathrin adaptors and found that the knockdown of specific clathrin components phenocopy *apg-1* and *apm-1* in nucleolar organization. This suggests that clathrin-mediated vesicle transport controls nucleolar organization. Subsequently, by performing germline-specific RNAi, I found that clathrin does not act in germline stems to regulate their nucleolar organization. Inspired by these results, I aim to determine from which tissue clathrin-mediated endocytosis regulates germline stem cell nucleolar organization, the molecular mechanisms involved in this process, and its physiological implications. This work will potentially provide new insight into somatic regulation of nucleolar and germline stem cell health.

299A Programmed DNA Elimination in *Auanema rhodense*

Tsz Wai Chan[1], Sally Adams[1], Liesl Grace Strand[2], Anne Villeneuve[2], Pablo Gonzalez de la Rosa[3], Mark Blaxter[3], Andre Pires da Silva[11]School of Life Science, University of Warwick, [2]Stanford University, [3]Wellcome Sanger Institute

In animals, usually the germline genome is set aside early in development and is protected from changes. In contrast, the somatic genome, which is not passed to the following generation, may undergo drastic remodelling. A prominent example is Programmed DNA elimination (PDE), a developmental process in which genomic regions in somatic cells are targeted for removal during embryogenesis. Although PDE has been reported in a variety of species, the precise spatiotemporal regulation, mechanisms, and biological significance of this process remains largely unknown.

In the free-living nematode *Auanema rhodense*, the somatic genome is much smaller (60 Mb) compared to the germline (180 Mb), indicating that *A. rhodense* undergoes substantial PDE during embryonic development. PDE in *A. rhodense* results in the removal of specific parts of the DNA across all chromosomes, potentially directed by a DNA sequence motif that marks regions for elimination.

Using fluorescence *in situ* hybridisation (FISH) and quantitative PCR, we determined that PDE occurs between the 6-20 cell stage of embryogenesis in *A. rhodense*. Furthermore, immunofluorescence staining of the histone markers indicates dynamic chromatin remodeling – characterized by the temporal recruitment of euchromatic (H3K36me3) and heterochromatic (H3K27me3) markers during anaphase and telophase – likely critical for facilitating the process. Additionally, transcriptomic analyses have identified candidate genes whose expression patterns and homology implicate them in the regulation of PDE.

Unravelling the specific mechanisms of PDE that occur in *A. rhodense* will not only enhance our knowledge of embryonic development in this species, but can also provide valuable insights for advanced genome editing applications in clinical and biotechnology settings.

300A Implication of the BORC complex in homeostatic regulation of germline stem cell proliferation in *C. elegans*

Armi Manharbhai Chaudhari[1], Minh Thu Nguyen[2], Alexandre Clouet[2], Patrick Narbonne[21]Department of medical biology, Universite du Quebec A Tois-Rivieres, [2]Department of medical biology, University of Quebec trois riveres

Stem cells undergo symmetric divisions for self-renewal or asymmetric divisions for differentiation, regulated by niche signaling. Growth factors primarily control stem cell proliferation and differentiation. In *C. elegans* hermaphrodites, the insulin/IGF-1 pathway promotes germline stem cell (GSC) proliferation based on nutrition, while the ERK/MAPK pathway, acting from the gut or somatic gonad, promotes proliferation when gametes are needed. When oocytes accumulate without sperm, MPK-1/ERK suppression reduces GSC proliferation. Using an *oma-1; oma-2* background to prevent oocyte activation and activate homeostatic signaling in young adults, we screened for mutants in which GSC proliferation kept going and led to the formation of differentiated germline tumors consisting of disorganized oocytes. This identified *kxd-1(nar55)*, a component of the BLOC-one-related complex (BORC), consisting of BLOS-1, BLOS-2, BLOS-4, BLOS-7, BLOS-9, SNPN-1, KXD-1, and SAM-4. RNAi knockdown of other BORC subunits phenocopied *kxd-1*, indicating BORC suppresses GSC proliferation. BORC interacts with ARL-8, a GTPase regulating lysosomal positioning. Knockdowns of *arl-8* and *ppk-3*, involved in lysosome maturation, phenocopied *kxd-1(nar55)*. Soma- and germline-specific *blos-9* rescue experiments confirmed BORC's primary role in the germline. While both SAM-4::mScarlet and ARL-8::mCitrine are cytoplasmic in wild-type germlines, ARL-8::mCitrine becomes nuclear in the absence of *sam-4*, corresponding to the ARL-8(T34N)::mCitrine loss-of-function variant. However, ARL-8::mCitrine remained cytoplasmic when other BORC subunits, including BLOS-9, BLOS-1, or BLOS-4, were mutated, suggesting ARL-8 activation depends specifically on SAM-4 or certain BORC subunits. Most interestingly, both SAM4::mScarlet and ARL8::mCitrine became predominantly perinuclear in *oma-1; oma-2* germlines, while ARL8::mCitrine remained cytoplasmic when BORC complex genes were mutated in the *oma-1; oma-2* background. While the BORC complex was viewed as being limited to lysosomal and synaptic vesicle positioning/transport, these results propose a new intriguing. We hypothesized that perinuclear BORC localization influence cytoplasmic export and/or translation. Supporting this, *sftb-1*, encoding a splicing factor, exhibited a phenotype similar to BORC mutants. The perinuclear localization of the BORC complex may represent a novel mechanism linking lysosomal regulation (PPK3) and RNA biogenesis (SFTB1) together to achieve homeostatic regulation of GSC proliferation. The high degree of similarity between *C. elegans* BORC complex subunits and their human counterparts leads us to believe that our work may provide valuable insights into the many rare diseases caused by BORC complex defects, as well as towards human stem cell regulation and cancer.

301A Non-autonomous insulin signaling delays mitotic progression in *C. elegans* germline stem and progenitor cells

Eric Cheng, Ran Lu, Abigail Gerhold Biology, McGill University

Proper chromosome segregation during mitosis is essential for maintaining genomic integrity in stem and progenitor cells. However, how these cells maintain mitotic fidelity within the complex physiological environment of a living organism remains poorly understood. To address this question, we used *in situ* live-cell imaging of *C. elegans* germline stem and progenitor cells (GSPCs) to investigate how mitotic progression and mitotic fidelity are affected by the activity of signaling networks and environmental conditions. We performed a candidate screen of signaling pathways known to regulate other aspects of GSPC behavior to identify those that impact the duration of GSPC mitosis. We found that reducing activity of the insulin receptor *daf-2* (*daf-2(e1370)*) delays GSPC mitosis. These delays act through the canonical, DAF-2/IGFR to DAF-16/FoxO, insulin signaling pathway; however, both DAF-2 and DAF-16 act cell non-autonomously, primarily from the intestine and somatic gonad. Mitotic delays in *daf-2(e1370)* GSPCs depend on the spindle assembly checkpoint but are not accompanied by overt defects in spindle assembly or increased chromosome segregation errors. This phenotype is distinct from what we observe when we alter environmental conditions by imposing caloric restriction, which delays GSPC mitosis but also increases the frequency of chromosome segregation errors. Correspondingly, we found that mitotic delays upon caloric restriction do not require the canonical insulin pathway but instead require *daf-18*/PTEN and *aak-1/2/*AMPK. Together, these findings demonstrate that at least two genetically separable signaling pathways regulate GSPC mitosis. DAF-2 to DAF-16 insulin signaling in the soma delays GSPC mitosis without compromising spindle assembly, possibly by strengthening the spindle assembly checkpoint, while DAF-18 and AMPK, either together or in parallel, delay GSPC mitosis in response to caloric restriction. Ongoing work is focused on exploring mechanisms that could delay mitosis without impairing spindle assembly in *daf2(e1370)* GSPCs, including increased spindle assembly checkpoint strength or delayed spindle assembly checkpoint silencing. Altogether, this work highlights the challenges stem and progenitor cells face in preserving mitosis in dynamic signaling and physiological environments *in vivo*.

302A Two *C. elegans* VCP Homologs, CDC-48.1 and CDC-48.2, are Upregulated During Oocyte Development and Respond to Germline Protein Stress

Md Helal Uddin Chowdhury, Joshua P. Gill, Alyssa E. Johnson, K. Adam Bohnert Biological Sciences, Louisiana State University - Baton Rouge

Valosin-containing protein (VCP) regulates protein homeostasis in multiple species and cell types, contributing to processes including ER-associated protein degradation and autophagy. Though VCP has some documented functions in the germline, whether it contributes to germline proteostasis is unclear. In the nematode *Caenorhabditis elegans*, oocyte maturation has been linked to proteostasis enhancement before fertilization. Here, we developed endogenous fluorescent knock-ins for two *C. elegans* VCP homologs, CDC-48.1 and CDC-48.2, to assess their potential relationship to this pathway. We found that endogenous CDC-48.1::mNG and endogenous CDC-48.2::mNG were strongly expressed in the *C. elegans* germline and showed developmental patterns of protein expression shaped by the translational repressor GLD-1 and MAP kinase MPK-1. While each mNG-tagged CDC-48 paralog was essentially undetectable at early to mid-pachytene stages within the adult hermaphrodite germline, they became detectable at the bend of the gonad arm and further increased in intensity in sperm-proximal maturing oocytes. At a subcellular level, CDC-48.1::mNG and CDC-48.2::mNG localized inside the nucleus and more broadly to punctate structures throughout the cytoplasm of wild-type oocytes. We performed a candidate screen to identify mutations that altered CDC-48.1::mNG localization. We found that a loss-of-function mutation in *atln-1*/Atlastin caused CDC-48.1::mNG to concentrate into a large mass in the cell, which overlapped with collapsed ER. We propose that this redistribution of CDC-48.1::mNG may be due to enhanced ER protein stress in this genetic background. Interestingly, we observed that CDC-48.1::mNG protein expression in oocytes increased above basal levels under separate conditions inducing oocyte protein stress; namely, upon knockdown of the lysosomal V-ATPase or upon loss of sperm signaling. These data suggest that *C. elegans* VCP homologs may be primed to respond to proteostasis dysfunction in germ cells, perhaps even redistributing to distinct sites of stress upon activation. We are currently evaluating whether the CDC-48-labeled punctate structures in wild-type oocytes represent ER sites or other structures, such as autophagosomes or lysosomes, and we are testing the necessity of the CDC-48 paralogs in *C. elegans* oocyte proteostasis enhancement.

303A Regulation of metalloprotease activity during apical extracellular matrix remodeling

John C Clancy Molecular, Cellular, and Developmental Biology, University of California, Santa Cruz

Specialized extracellular matrices cover the apical surface of all epithelial cells, acting as a barrier to pathogens and environmental insults. The *C. elegans* cuticle is a highly dynamic apical extracellular matrix (aECM) that is continuously remodeled. *C. elegans* progress through four larval stages before reaching adulthood, where each larval stage is terminated by a molt and the animal replaces its old aECM with a new one. The remodeling of the aECM is facilitated by proteases and their activity is tightly regulated to ensure aECM functionality. One way to achieve this regulation is through the action of endogenous protease inhibitors. However, how protease inhibitors regulate the activity of proteases at the right time and place to ensure proper aECM remodeling is largely unknown. Animals with mutation to *bli-5*, a putative protease inhibitor, exhibit a blistered cuticle while animals with mutation to *nas-37*, an astacin metalloprotease, are unable to properly shed their old cuticles. My preliminary data show that knockdown of *nas-37* in *bli-5* mutants suppresses the blister phenotype caused by mutation to *bli-5*, suggesting the proteins encoded by these genes could function in concert. However, knockdown of *nas-37* does not suppress the blister phenotype in other *bli* mutants, suggesting that the interaction between *nas-37* and *bli-5* is specific. Additionally, my preliminary data shows that the medial layer of the cuticle is present and BLI-1::mNG punctae are restored when *nas-37* is knocked down in *bli-5* mutants. To further understand the role of *bli-5* and *nas-37* in aECM remodeling, I introduced endogenous fluorescent tags and deletion alleles using CRISPR/Cas9. NAS-37 is secreted and is enriched in the head of the animal which is consistent with its role in the degradation of the old cuticle. Additionally, BLI-5 is secreted into the cuticle and trafficked through the lysosomes. My current studies are focused on determining the nature of the interaction between *bli-5* and *nas-37* and providing a bigger picture understanding of the complex regulatory pathways that ensure proper aECM remodeling and functionality.

304A Defining the Molecular and Physiological Consequences of the Loss of miRNA Mediated Regulation of the *C. elegans* Notch Receptor *lin-12*

Eric Cormack, Luisa Cochella, Emilio Santillan Molecular Biology and Genetics, Johns Hopkins School of Medicine

The C. elegans miR-51 family is deeply conserved across a wide range of animals and is essential for C. elegans development (Alvarez-Saavedra & Horvitz, 2010; Shaw et al., 2010). Among a number of validated targets (E.S. and L.C. unpublished work), miR-51fam is predicted to target the Notch receptor *lin-12* via two predicted high-affinity binding sites that are highly conserved across a range of nematodes. Using endogenous reporters (Pani et al., 2022) and CRISPR mutagenesis we demonstrate that miR-51 functions as a negative regulator of LIN-12 receptor dosage in multiple LIN-12 dependent developmental processes. We hypothesized that this increase in LIN-12 expression would lead to corresponding 'gain of function' phenotypes associated with *lin-12(d)* mutations (Greenwald and Seydoux, 1990), or loss of negative regulators of LIN-12 (Hubbard, et al., 1997; Sundaram and Greenwald, 1993). We disentangled the effect of miR-51fam regulation of *lin-12* from its effect on other targets by generating strains in which we mutated one or both miR-51 binding sites in the *lin-12* 3' UTR (*lin-12BSM*). Thus far, functional genetics experiments and live imaging of well characterized LIN-12 dependent processes, including the AC/VU decision and VPC patterning, have failed to reveal obvious Notch 'gain of function' phenotypes in animals carrying the *lin-12BSM* allele. Nevertheless, we have observed several molecular defects and low penetrance phenotypes related to vulva development in these animals that we aim to understand. Moreover, animals with the *lin-12BSM* allele do display a penetrant and expressive sterility, which is exacerbated at 25ºC, although the focus of action of LIN-12 related to this phenotype is unclear. By continuing to measure the molecular consequences of increased LIN-12 expression, as well as more broadly studying the developmental consequences of this increase, we hope to gain insight into the need for dosage control of LIN-12 and the conservation of this regulation by miR-51 in nematodes, and possibly by other miRNAs in other animals.

305A A conserved insulin signaling pathway in the parasitic nematode *Brugia malayi*

Kirsten Crossgrove, Sarah E Ferguson, Lauren M Dethridge, Benjamin L Skaleski, Libby C Nabhan, Miles A Hagen Biology, Univ Wisconsin, Whitewater

The parasitic nematode *Brugia malayi* causes lymphatic filariasis in humans. The dauer hypothesis postulates that the infective (iL3) stage parasites, which are transmitted from mosquitoes to humans during a blood meal, share similarities with the dauer stage in *C. elegans*. Specifically, both iL3 and dauer are third stage larvae that are arrested until a change in environmental conditions triggers molting to the L4 stage. For dauer, that change is signaled by the presence of food, less crowding and/or lower temperatures, while for the parasite it is the transfer to the human host. The insulin/IGF-1 signaling pathway (IIS) is known to regulate dauer formation and recovery in *C. elegans*, and we hypothesize that it works similarly to regulate iL3 in *B. malayi*. We have generated eukaryotic cell culture expression constructs for several *B. malayi* IIS genes, including *Bma-daf-16*, *Bma-pdk-1*, *Bma-akt-1, Bma-pptr-1,* and *Bma-ftt-2*. We are using cell culture assays to determine whether the parasite proteins have similar function to *C. elegans* and mammalian IIS proteins. Previous experiments in our lab showed that *Bma*-DAF-16, the predicted downstream target of the IIS pathway, can activate expression of a firefly luciferase reporter gene controlled by six DAF-16 binding sites. We show evidence that the ability of *Bma*-DAF-16 to activate reporter gene activity can be altered by co-expression of other IIS pathway proteins in cell culture. We discuss the utility of mammalian cell culture as a model for parasite IIS pathway function.

306A Investigating P granule function in *Caenorhabditis* nematodes

Andrew Delvoye, Sumaiya Chowdhury, Lisa N Petrella Marquette University

P granules in *Caenorhabditis elegans* protect against temperature-induced sterility. However, it has yet to be discovered if the function of P granules is conserved in the closely related species in *C. briggsae*. In *C. elegans*, *glh-1, mip-1,* and *pgl-1* mutants are more sensitive to temperature stress, becoming completely sterile at 26oC, while wild-type *C. elegans* become sterile at 27oC. Wild-type *C. briggsae* become sterile at 30oC, so if the function of P granules is conserved, we would expect in *C. briggsae* P granules mutants will experience sterility at 29oC. Here, we hypothesize that P granules in *C. briggsae* are used to protect against heat stress, as found in *C. elegans*. We will test our hypothesis using RNAi to knock-down *pgl-1*, *mip-1*, and *glh-1* in *C. briggsae*. We will assess fertility in *C. briggsae* with RNAi knock-down of each P granule component at non-stress (20oC) and stress temperatures. We will also assess the structure of the P granules in *C. briggsae* at non-stress and stress conditions (29oC). Learning more about the conservation of P granules function will allow us to understand how rising temperatures may affect organisms and the ecosystems they rely on. It will also give us insight into the mechanisms used by *C. briggsae* to protect against rising global temperatures.

307A LEM-3/Ankle1 nuclease prevents the formation of syncytium and safeguards neuronal differentiation in *C. elegans*

Siyu Deng, Chaogu Zheng The University of Hong Kong

Ontogenesis of multicellular organisms from zygotes to adults involves multiple rounds of cell fate determination. The fundamental question of how cells acquire their specific identities has intrigued researchers for centuries. Once a cell commits to a specific fate, it follows a predefined developmental pathway that is often resistant to fate alteration at later stage of differentiation. Various factors play crucial roles in this intricate process of cell fate decision, yet the contribution of DNA endonucleases to this process has been relatively understudied.

This study delves into the functional role of the LEM-3 endonuclease in regulating cell fate determination in terminally differentiated neurons. Using *C.elegans* as an *in vivo* model to investigate the fate determination process of post-mitotic cells, we found that the loss of *lem-3* resulted in the emergence of additional cells that exhibited a touch receptor neuron (TRN)-like fate and were connected with the original TRNs via intercellular canals. These extra cells are the sister cells of TRNs and form binuclear syncytium with the TRNs due to failure of cytokinesis at the last round of cell division. Intriguingly, in some cases, the two nuclei in the syncytium can carry out distinct transcriptional programs, suggesting that connected neurons could still maintain certain levels of autonomy in transcriptional regulation.

At the translational level, the formation of intercellular canals facilitates the exchange of various types of proteins that contribute to cellular differentiation. As a result, although the transcriptional programs of the two connected cells in the syncytium can be independent, their cellular content are mostly mixed. The absence of LEM-3 disrupted proper resolution of chromatin bridges during anaphase, leading to the formation of intercellular canal. The enzymatic activity of LEM-3 is important for its regulatory role in cell fate determination. The canal can grow and persist into late adult stages, suggesting a cytoskeleton-dependent maintenance mechanism.

LEM-3 also regulates the fate determination of other cell types. By analyzing several examples of fate decision between sister cell pairs, we found that LEM-3 plays a role in the fate choice between apoptotic and non-apoptotic cells, between two distinct neuronal fates, and between neuronal and non-neuronal fates.

In conclusion, this research revealed a previously underappreciated function of LEM-3/ANKLE1 endonuclease in safeguarding cellular differentiation by processing unresolved DNA connections during mitosis and enabling cytoplasmic separation. Our analysis of the mechanisms underlying intercellular canal formation and persistence contribute to the understanding of the repercussions of sustained connections between post-mitotic sister cells.

308A Genetic interactions impacting sperm competence and oocyte maturation

Anita Fernandez, Kaitlin Levangie, Gabriela Vida, Aura Cristina Spar, Giulia Crosio, Julia Stobierska, Sydney Youd, Anna Weissenberg, Caitlin Fanella, Kaitlyn Santos, Serena Koshy Biology, Fairfield University

We have been studying low-fecundity mutants in order to understand how microtubule motors and their regulators contribute to the formation of functional gametes in *C. elegans*. The *dnc-1* gene encodes the p150 Glued subunit of dynactin, which is an activator of microtubule motors. At 26 °C, *dnc-1(or404ts)* mutants produce a small brood size and pass many apparently-unfertilized oocytes. We observed several defects suggesting sperm problems in *dnc-1* mutants. For example, *dnc-1* male sperm fail to outcompete wild-type hermaphrodite sperm. Also, *dnc-1* mutant male sperm have small pseudopods. Self-fertilizing *dnc-1* mutant hermaphrodites deplete their sperm faster than wild-type hermaphrodites. These observations support the idea that defects to dynactin impact sperm function, possibly by impairing sperm motility. All the sperm defects observed are partially rescued by mutations in Y-complex nucleoporin genes *mel-28* and *npp-5*. This suggests a novel role for these proteins in sperm, which do not have nuclear envelopes.

While further investigating roles for MEL-28 outside the nuclear pore, we generated *dhc-1 (or283ts); mel-28(t1684)* double mutants. *dhc-1* encodes the largest subunit of the minus-end-directed microtubule motor dynein. At 26 °C, *dhc-1(or283ts)* single-mutant hermaphrodites and *mel-28 (t1684)* single-mutant hermaphrodites produce many embryos. *dhc-1; mel-28* hermaphrodites, in contrast, produce few eggs. This synthetic low-fecundity defect suggests that dynein and MEL-28 act in parallel to promote fertility. Several observations support the idea that *dhc-1; mel-28* double mutants have oocyte formation defects. Mating N2 males to *dhc-1; mel-28* double-mutant hermaphrodites does not rescue the low brood size, and *dhc-1; mel-28* double have a disorganized proximal gonad with small round oocytes. To test if the double mutants have a yolk uptake defect, we crossed the YP170::tdimer2 transgene into the double mutants. Yolk is produced by the intestine, secreted into the pseudocoelom, and then received by maturing oocytes via receptor-mediated endocytosis. The single mutants do not have a yolk uptake defect. However, in the double mutants, the tagged yolk protein accumulates in the pseudocoelom and is not imported into the oocytes. This suggests that MEL-28 and dynein work together in yolk import. Our work suggests that MEL-28, a well-characterized nucleoporin, plays a role in membrane fusion events needed in both oocytes and sperm.

309A *mpk-1* promotes germline stem cell proliferation from the soma via direct stimulation of cytidine deaminases

Lloyd Venceslas Fotso Dzuna[1], Patrick Narbonne[21]Biologie médicale, Université du Québec à Trois-Rivières, [2]Université du Québec à Trois-Rivières

Adult stem cells respond to the needs for differentiated cells in the tissues they maintain, proliferating to replace old or damaged cells with new ones in order to preserve tissue integrity. This applies to the *C. elegans* germline, where the absence of sperm and the accumulation of unfertilized oocytes lead to a reduction in MPK-1/ERK signaling and suppression of germline stem cell (GSC) proliferation. Since MPK-1 can non-autonomously promote proliferation from the intestine or gonadal sheath, we first determined whether the shutdown of *mpk-1* signaling is required in both tissues for feminization to induce a reduction in GSC proliferation. We expressed the gain-of-function allele *let-60(ga89)* specifically in these tissues in feminized animals to ectopically activate *mpk-1* signaling. Our results indicated that *mpk-1* signaling must be inhibited in both tissues for feminization to suppress GSC proliferation. Next, we wondered how MPK-1 activity in the intestine could affect GSC proliferation from a distance. Chi et al. (2016) showed that nucleoside levels could affect GSC proliferation when the diet was low in uridine and/or thymidine, such as the one provided by OP50 E. coli. Two cytidine deaminases (CDD) genes present in the *C. elegans* genome, *cdd-1* and *cdd-2*, are redundant, and animals lacking these two genes had low GSC proliferation, similar to animals lacking *mpk-1*. Moreover, as with MPK-1, CDD-1/2 activity was required either in the intestine or somatic gonad to promote GSC proliferation (Chi et al. 2016). Interestingly, we identified a potential MPK-1 consensus phosphorylation motif on CDD-1/2, corresponding to CDD-2 serine 93 (S93). We generated a phosphomimetic version of *cdd-2(S93D),* which prevented the downregulation of GSC proliferation in feminized animals. Strikingly, *cdd-2(S93D)* restored GSC proliferation and sperm formation in *mpk-1* null mutants. As a control, we also generated a non-phosphorylatable version of *cdd-2(S93A)* that did not perturb GSC proliferation. It is believed that the insulin/IGF-1 pathway promotes GSC proliferation in collaboration with MPK-1 signaling. Interestingly, the expression of *cdd-2(S93D)* also restored GSC proliferation in *daf-2(e1370)* mutants, while proliferation remained unchanged when *cdd-2(S93A)* was used instead. Thus, c*dd-1/2* may promote GSC proliferation downstream of both pathways. We next want to understand how these small molecules that *cdd-1/2* synthesize move from the soma to the germline.

310A Targeting cyclic Nucleotide Phosphodiesterases as potential nematicides using chemical and molecular agents

Kranti K Galande, Riley M Wilson, John J Collins, Rick H Cote Molecular,Cellular and Biomedical Sciences, University of New Hampshire

There are nearly 4100 species of plant parasitic nematodes that cause a serious constraint for global food security, and the estimated crop damage due to plant parasitic nematodes is about ~$100B annually. Phosphodiesterases (PDEs) are major determinants of cellular levels of cyclic nucleotides and are the only enzymes capable of hydrolyzing the two second messengers, cAMP and cGMP. Nematode behavior and lifecycle depend on cyclic nucleotide signaling, but currently there is limited knowledge of the role individual PDEs play in mediating *C. elegans* signaling pathways. Mammals possess 11 PDE families and nematodes have six PDE genes representing six of these families. To evaluate, whether the disruption of one or more PDEs will disrupt nematode behavior, we employed several strategies; 1) exposure of *C. elegans* to family-specific inhibitors designed to target human PDEs; 2) creation of *C. elegans* strains in which each of the six individual PDE genes was ablated using CRISPR/Cas9; 3) use of RNA interference (RNAi) to disrupt *C. elegans* PDEs *in vivo*; and 4) creation of double “knockout” strains by genetic crosses. Interestingly, none of the single knockout strains showed major defects in development or reproductive success. Of the six knockout strains, only the *pde-1-/-* nematodes were defective in their chemotactic responses, as were wildtype *C. elegans* exposed to the human PDE1 inhibitor, PF-04471141. The *pde-1-/-:pde-4-/-* strain exposed to *pde-3* RNAi and the *pde-1-/-:pde-3-/-* strain exposed to *pde-4* RNAi both showed significant reductions in fecundity, developmental delays, and lethargic phenotypes. We conclude that many physiological and behavioral phenotypes observed in *C. elegans* rely on multiple PDE family members to regulate cyclic nucleotide signaling pathways, and that nematode PDEs represent a promising target for developing chemical or genetic controls to disrupt the lifecycle and/or food-sensing abilities of plant parasitic nematodes, thereby enhancing crop productivity and food security.

311A Control of cell-cycle transitions by heterochronic factor LIN-14 in the *C. elegans* intestine

Daniel Iglesias van Montfort, Tess de Kleijn, Semih K Aslan, Matilde Galli Hubrecht Institute

Multicellular development requires precisely timed cell divisions to ensure the formation of tissues and organs with characteristic architectures and sizes. More than 40 years ago, heterochronic mutants were described in *C. elegans*, in which the timing of larval-stage divisions is altered. Despite extensive interest in the heterochronic pathway, most studies have focused on the role of microRNAs and RNA binding proteins in the pathway, and it remains unknown what the downstream targets are of the heterochronic transcription factors that control cell-cycle behavior. In our group we study how the heterochronic transcription factor LIN-14 controls the cell cycle using the intestinal lineage as a model system, in which cells undergo characteristic cell-cycle transitions during defined moments of larval development. By performing intestine-specific degradation of endogenously tagged LIN-14::sfGFP::AID at different moments in development, we find that LIN-14 function is required within the first eight hours after hatching to ensure that cells enter S phase and become binucleated at the end of the L1 stage. Intriguingly, we see that overexpression of LIN-14 during different moments in development gives rise to different cell-cycle phenotypes: overexpression during early L2 stage can result in tetranucleated instead of binucleated intestinal cells, but this phenotype is not observed if LIN-14 is overexpressed during the L1 or L3 stage. To better understand how LIN-14 controls the cell cycle, we performed RNA sequencing on isolated intestinal cells after depletion of LIN-14. We identified many genes that were more highly expressed in the absence of LIN-14, suggesting that LIN-14 mostly acts as a repressor in the intestine. By cross-referencing our RNA-seq data to publicly-available LIN-14 ChIP-seq data, we identified 14 cell-cycle regulators that could be direct targets of LIN-14, including *efl-1*, *cdk-1* and *cdc-25.2*, which are well-known regulators of the G1/S and G2/M transitions, respectively. We are currently testing whether mutation of LIN-14 binding motifs in these genes can recapitulate the LIN-14 depletion phenotypes that we observe. Taken together, our results suggest that LIN-14 may play multiple roles in intestinal cell-cycle progression. In future work we hope to elucidate whether LIN-14 directly targets specific cell-cycle regulators to control cell-division patterning, and how LIN-14 is able to drive different cell-division patterns in different cell types.

312A Determining the essential roles of CRM-1 (cysteine rich transmembrane BMP regulator 1) in the absence of BMP signaling

Dillon M Gardner, Julia Chen, Fu-Yu Tsai, Jun Liu Molecular Biology and Genetics, Cornell University

The highly conserved Bone Morphogenetic Protein (BMP) signaling pathway is critical for metazoan development and homeostasis. In *C. elegans,* BMP signaling is not essential for viability, but is known to regulate several processes, including body size and mesoderm patterning. We have found that loss of function of the *crm-1* gene led to a synthetic lethal phenotype when combined with loss of BMP signaling. *crm-1* (cysteine rich transmembrane BMP regulator 1) encodes a conserved protein that contains multiple von Willebrand factor type C (vWC) domains, which are known to have BMP binding activity in vitro. Using several *crm-1* knockout alleles we generated via CRISPR, we found that *crm-1(0)* null animals showed no obvious BMP mutant phenotypes in body size and mesoderm patterning. However, *crm-1(0)* exhibited novel genetic interactions with BMP pathway mutants in a dosage-dependent manner: double mutants between *crm-1(0)* and mutations in core pathway components were synthetic lethal, while double mutants between *crm-1(0)* and mutations in genes that positively affect BMP signaling exhibited significant decreases in body size and viability. This synthetic lethality suggests that CRM-1 functions in some other signaling pathway(s) in a manner that is essential only in the absence of BMP signaling. We have tagged the endogenous CRM-1 with AID(AUXIN INDUCIBLE DEGRON)::GFP::3xFLAG using the CRISPR-Cas9 system. We are conducting experiments to determine where different isoforms of CRM-1 are expressed and localized and identify when and where their activity is essential in the absence of BMP signaling. Results from these experiments will inform hypotheses on how CRM-1 functions as well as how the BMP signaling pathway interacts with other signaling pathway(s) to ensure proper development.

313A ZTF-30: a transcription factor required for coordinated dauer stage remodeling

Luciana F Godoy[1,2], Eugenia Paez[1], Judith L Yanowitz[3], Daniel Hochbaum[11]CEBBAD, Universidad Maimonides, [2]Universidad de Buenos Aires, [3]Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute

Caenorhabditis elegans exhibit remarkable whole-organism developmental plasticity when exposed to harsh conditions as L1 larvae. In response to heat, starvation, and crowding, they arrest reproductive growth and enter a diapause stage called dauer. Significant tissue remodeling of the muscle, hypodermis, intestine, and nervous system allows for body constriction, secretion of specific collagens to form an impermeable cuticle, reduction of metabolic rate, and alterations in sensory and locomotive behavior. Dauer formation can be induced either by sensitized genetic background, exposure to dauer pheromone, or cultivation in liquid media under starvation conditions. In all cases, a loss-of-function mutation in the zinc finger transcription factor *ztf-30* results in partial dauers that fail to survive 1% SDS treatment, a hallmark of the dauer stage. Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), we determined that *ztf-30* mutant dauers exhibit defective cuticle deposition. The cuticle phenotype may be explained by one of two alternatives, either the hypodermis of *ztf-30* mutants is refractory to the dauer-inducing cues or the dauer genetic program in the hypodermis is not executing properly. Commitment of the hypodermis to dauer fate is confirmed by the expression of the *col-183p* reporter, suggesting that the hypodermis is responsive and that functional *ztf-30* is necessary to acquire dauer-specific cuticle traits. Interestingly, when examining the expression pattern of *ztf-30::GFP*, we found that signal is only detected in a single head neuron. Additionally, using two different transcriptional fluorescent reporters, we found that *ztf-30* positively regulates the DBL-1/BMP pathway, a TGF-β-related pathway in *C. elegans* known to regulate collagen expression in the hypodermis. Downregulation of the DBL-1/BMP pathway through RNAi feeding mimics the partial dauer phenotype observed in *ztf-30* mutants. Our work identifies *ztf-30* as a transcriptional regulator that uncouples the dauer entry decision from the dauer morphogenesis of the cuticle. We also show that perturbation of the DBL-1/BMP pathway results in partial dauers and suggest that the *ztf-30* abnormal dauer phenotype is explained by DBL-1/BMP downregulation.

314A BUB-1 functions post-mitotically in the epidermis to drive embryonic elongation

Rebecca Green[1], Neha Varshney[2], Noura Yassir[2], Taekyung Kim[3], Karen Oegema[2], Arshad Desai[21]Biology, UCSD, [2]UCSD, [3]Pusan National University

Morphogenetic processes shape the body plans of developing organisms across Metazoa. In *C. elegans*, elongation transforms the initially oval-shaped embryo into an elongated larval form. Early elongation is thought to be driven by differential contractility in the lateral versus dorsal/ventral epidermis, although the underlying mechanisms remain an active area of investigation. Here, we investigate an unexpected new player in elongation: the kinetochore component BUB-1. BUB-1 is best known for its mitotic roles in spindle checkpoint signaling and chromosome segregation. Our discovery of a post-mitotic role for BUB-1 in elongation emerged from a striking phenotypic difference between two separation-of-function BUB-1 mutations, one disrupting the binding site for the anaphase regulator CDC20 and the second disrupting an adjacent docking site for the mitotic kinase PLK-1. In the early embryonic divisions, both mutants impair CDC-20 recruitment, preventing kinetochores from properly controlling anaphase timing, without causing defects in chromosome segregation. However, the PLK-1 docking site mutant—but not the CDC-20 binding site mutant—leads to a severe embryo elongation defect. These results suggested that BUB-1 has a role in embryonic elongation independent of its function in chromosome segregation and CDC-20 recruitment, potentially through its interaction with PLK1 kinase. Supporting this idea, BUB-1 and PLK-1 co-localize throughout elongation. To identify the specific tissue in which BUB-1 functions to promote elongation, we developed a set of heritable extrachromosomal arrays for tissue-specific expression of TIR1(F79G), the plant F-box protein that allows auxin-dependent degradation of target proteins. Arrays were introduced into strains in which sequences encoding GFP alone (as a control) or GFP fused to an auxin-inducible degron (AID) were inserted into the endogenous *bub-1* locus. Our results showed that auxin-mediated degradation of BUB-1 in the epidermis (skin) mimicked the BUB-1 PLK-1 docking mutant phenotype, causing an elongation defect with embryo arrest at the 1.5 to 2-fold stage. Efforts to determine if BUB-1 functions in the lateral versus dorsal/ventral epidermis and to elucidate which aspects of BUB-1's multiple molecular interfaces function in the skin to promote elongation are underway. Collectively, this work defines a post-mitotic role for BUB-1 in embryonic morphogenesis and has the potential to advance mechanistic understanding of embryo elongation.

315A Molecular and genetic interactions between the DBL-1/BMP signaling pathway and BLMP-1/BLIMP1 regulate organismal traits in *Caenorhabditis elegans*

Tina L. Gumienny[1], M. Farhan Lakdawala[2], Matt Crook[31]Biology, Texas Woman's University, [2]AbbVie, Inc., [3]Department of Biology, Wofford College

Bone morphogenetic protein (BMP) signaling helps orchestrate multiple organismal traits in animals by regulating target gene expression. Regulation of this signaling pathway is critical for normal development and homeostasis. However, understanding how this pathway's transcriptional regulators, called Smads, control target gene expression to generate different traits is not well understood. Using the *C. elegans* system, we identified the chromatin remodeler B-lymphocyte maturation protein-1 (BLMP-1) as a partner in Smad-mediated gene expression regulation. BLMP-1 controls organismal traits that the DBL-1/BMP pathway also affects. While body size *blmp-1* mutants were epistatic to DBL-1 pathway mutants for male tail development, hermaphrodite gonad development, brood size, movement, and survival traits. DBL-1 signaling and BLMP-1 transcriptionally regulate each other. DBL-1 pathway Smads and BLMP-1 physically interact and regulate expression of common downstream target genes. This work identifies novel interactions between the DBL-1 signaling pathway and BLMP-1, two conserved major transcriptional regulators, that ultimately influence a spectrum of organismal traits. We propose that BLMP-1 regulates BMP signaling by acting as a gatekeeper, remodeling the chromatin architecture to permit the Smad complex access to target genes.

316A Identification of a new gene important to sperm activation in *C. elegans*

Anushree Suvrutt Gurjar, Maliyah Long, Amber Krauchunas Biological Sciences, University of Delaware

For successful fertilization it is important to ensure that a sperm develops and functions correctly. The development and function of sperm is regulated by the genes involved in spermatogenesis, spermiogenesis and fertilization. While in *C. elegans* several genes necessary for these processes have been identified, we still don't know all the genes involved in the development and function of sperm. The purpose of this study is to determine whether the sperm-specific transcript *F57A8.6* is important to sperm function and development. To test this, we created a knock-out mutant of *F57A8.6* and performed phenotypic characterization. We observed that knocking out *F57A8.6* results in significantly reduced hermaphrodite self-fertility. To investigate the reason for reduced fertility, we DAPI stained hermaphrodites to quantify the number of sperm nuclei in the spermathecae. We observed that the number of sperm produced by *F57A8.6* hermaphrodites is identical to wildtype levels but there is significantly less sperm present in *F57A8.6* hermaphrodites compared to wildtype after several rounds of ovulation. This led us to conclude the mutant sperm suffer from localization defects. To assess the underlying cause of the localization defect, we dissected sperm from *F57A8.6* hermaphrodites, and observed that the otherwise normal spermatids do not undergo sperm activation to form motile activated sperm. Sperm activation is a tightly regulated process that transforms non-motile unactivated spermatids to form motile activated sperm. To further analyse the sperm activation defect, we tested whether the spermatids are capable of activating. When we exposed them to an in vitro activator, we observed that the spermatids were capable of forming activated sperm. We also tested this in vivo and observed that exposure to male seminal fluid led to an increase in the *F57A8.6* hermaphrodite self-fertility. This suggests the *F57A8.6* mutant sperm have all the right components to undergo activation but lack the ability to respond to the activation signal. Interestingly, knocking out *F57A8.6* renders a hermaphrodite-specific fertility defect; male fertility and male sperm are unaffected. In conclusion, we have identified a new gene important to sperm activation which acts in a unique sex-specific manner. Future work to place this gene in the context of the two known sperm activation pathways will provide further insights into the sex-specific differences of sperm activation in *C. elegans*.

317A Light exposure affects mouth-form plasticity through oxidative stress in *Pristionchus pacificus*

Hirokuni Hiraga[1], Ageha Onodera[1], Chinatsu Kai[2], Kenichi Nakayama[3], Keimei Ko[1], Takahiro Chihara[1], Misako Okumura[11]Graduate School of Integrated Sciences for Life, Hiroshima University, [2]School of Science, Hiroshima University, [3]Department of Cell Biology and Center for Cell Dynamics, Johns Hopkins University School of Medicine

Environmental cues profoundly shape organismal development, with light being one of the most ubiquitous and evolutionarily conserved signals. However, the molecular mechanisms linking early-life light exposure to distinct developmental outcomes remain largely unknown. This study investigates how light exposure during larval development influences phenotypic plasticity in the nematode *Pristionchus pacificus*, focusing on its well-characterized mouth-form dimorphism. The eurystomatous (Eu) form has a wide mouth opening and two tooth-like denticles, whereas the stenostomatous (St) form is characterized by a narrow opening and a single denticle. This polyphenism is determined by environmental conditions experienced during the larval stages, resulting in an irreversible decision to develop into either the Eu or St form as an adult.

We found that exposure to short-wavelength light during mid to late larval stages significantly increases the Eu form frequency. To elucidate the molecular mechanisms underlying this light-dependent plasticity, we analyzed mutants in the cGMP-dependent phototransduction pathway and mouth-form regulatory switch genes. Mutations disrupting the cGMP-dependent phototransduction pathway abolished the light-induced increase in the Eu form, and epistasis analysis placed this pathway upstream of the mouth-form switch gene. Our findings revealed that light signals integrate into the mouth-form switch gene network, which is required to elicit the Eu-biased phenotype. Transcriptome analysis showed that light exposure upregulates genes involved in xenobiotic metabolism and oxidative stress responses, while downregulating the sulfotransferase *sult-1*, which functions as the switch gene in mouth-form plasticity. We confirmed ROS involvement through multiple lines of evidence: (1) increased protein oxidation in light-exposed worms; (2) treatment with ROS-generating compounds induced the Eu form to a similar extent as did light exposure; (3) antioxidant treatment suppressed the light-induced effect; and (4) CRISPR-mediated knockout of ROS-scavenging genes increased Eu form development even under dark conditions. These results suggest that light exposure induces ROS, driving the developmental shift toward the Eu form. Our research provides insights into the interplay between environmental factors, stress physiology, and developmental plasticity, with broad implications for understanding how organisms adapt to variable habitats.

318A Implication of the HSP110/70/40 chaperone complex in germline protein aggregate management and developmental control

Emily E Hirsch, Amy Clemons, Bryan T Phillips Biology, University of Iowa

Amyloid proteins are historically implicated in many diseases of aging, such as Alzheimer's Disease and Huntington's Disease. However, functional protein aggregation is arising as a novel concept within developmental biology. Endogenous protein aggregates are present within *C. elegans* and have been identified as amyloid in nature. The role of endogenous protein aggregates in development remains unclear. Protein aggregation is typically regulated by a network of disaggregase enzymes along with chaperone proteins. Here, we test the developmental role of a trimeric complex of heat-shock proteins composed of Hsp110, Hsp70, and Hsp40/DNAJ proteins previously implicated in managing aging stress. In *C. elegans*, these proteins are HSP-110, HSP-1, and several members of the DNAJ protein family, respectively. We aim to understand how this complex regulates endogenous aggregates in *C. elegans* and their role in proper development. Viability assays utilizing L4 RNAi knockdown of HSP-1 yielded a severe decrease in brood size and surviving *hsp-1(RNAi)* progeny arrested growth at early larval stages. Complete sterility results by 48h on *hsp-1(RNAi)* compared to control cohorts that remain fertile. Loss of early larval HSP-1 caused larval arrest. *hsp-110* deletion worms develop normally during early larval stages but arrest as late larvae or early adults and are mostly sterile. Given the sterility phenotype of *hsp-1* and *hsp-110* loss, we examined the presence and material state of germline aggregates using the A11 antibody amyloid marker. A11 positive protein aggregates were observed in both mature oocytes as well as the rachis, as well as in arrested germlines observed with loss of HSP-1. To identify possible aggregating proteins and their associated membraneless organelles, we screened for colocalization with markers of known aggregation-prone condensate components. P-granules marked by PGL-1 showed little overlap with amyloid aggregates while RNA Processing bodies (P bodies), marked by CAR-1, showed enrichment in aggregated puncta. Investigation with other markers of membraneless organelles such as MEX-3 (P-granules), PAB-1 (P-granules and P-bodies), and MEG-1/3 (germline-specific P-bodies), along with RNA probing will inform the composition and functional status of observed protein aggregate structures. These data suggest that the HSP110 and HSP70 operates during development to promote proteostasis and is required for normal larval progression and germline function.

319A Developing genetic markers for neuronaliIdentification in *Pristionchus pacificus*

Ray Hong[1], Dylan Castro[2], Ivan Dimov[2], Heather Carstensen[21]Biology, Cal State U Northridge, [2]CSUN

The development of genetic markers for identifying neurons is critical for assigning homology and function in comparative studies. In the entomophilic nematode *Pristionchus pacificus*, several methods are available for gene expression studies in whole animals. Promoter fusion reporters can be maintained as complex extrachromosomal arrays and have the advantage of showing neurite expression in living specimens to narrow down possible neuronal homologs in *C. elegans* based on conserved neuronal morphology. However, low expression or non-faithful reporter expression and high incidences of reporter toxicity in *P. pacificus* have hindered our ability to systematically characterize gene expression from entire gene families. Furthermore, CRISPR/Cas9-mediated HDR insert size limit is less than RFP or GFP in *P. pacificus*, preventing the knock-in of reporter genes into loci of interest. To complement reporter expression studies, RNA fluorescent in situ hybridization using the recently developed Hybridization Chain Reaction (HCR) can provide high signal-noise ratio on fixed specimens and multiplex with several probes simultaneously. Some genes however, notably those coding for transcription factors, have been refractory to HCR so we instead have relied on protein tagging with the ALFA nanobody. The ability of GFP and RFP reporter proteins to remain intact after fixation allows for HCR and ALFA staining to be performed on well-characterized reporter strains to confirm spatial and temporal co-localization of promoter activity and mRNA expression. We use the identification of AFD, ASEL, and ASER markers to demonstrate the feasibility and ongoing challenges in using these three gene expression technologies to study neuronal patterning in *P. pacificus.*

320A Canonical and non-canonical Hippo signaling during development

Linh Huynh[1], Razan Fakieh[1,2], Cbrionne Hendrix[1], David J. Reiner[1,3]1College of Medicine, Texas A&M University, [2]Clinical Laboratory Sciences, Imam Abdulrahman bin Faisal University, [3]Institute of Biosciences and Technology

The Hippo pathway is a conserved signaling cascade crucial for tissue homeostasis and regeneration; its dysregulation can lead to cancer. In canonical Hippo signaling, the Hippo S/T kinase (MST1/2 in mammals) phosphorylates Warts S/T kinase (LATS1/2 in mammals), which in turn phosphorylates Yorkie/YAP, preventing its nuclear translocation and activation of Sd/TEAD-dependent transcription. Despite the widespread use of C elegans to investigate major developmental signaling pathways, thus far only YAP-1 repression by WTS-1/LATS has been characterized. We expanded on previous research by developing novel tools to investigate Hippo signaling. Using CRISPR/Cas9, we inserted sequences encoding a fluorescent protein into the 3' end of endogenous yap-1, creating an in vivo reporter (YAP-1::mNG) of upstream signaling activity. Deleting cst-1 and cst-2, tandem orthologs of Hippo/MST1/2, resulted in viable animals with nuclear YAP-1::mNG in hypodermal but not intestinal cells. In contrast, wts-1/LATS loss conferred L2 arrest with continued pumping, a phenotype reversible by yap-1- or egl-44/TEAD-directed RNAi, and led to nuclear YAP-1::mNG in both hypodermal and intestinal cells. Surprisingly, cst-1/2 deletion did not phenocopy wts-1 deletion in causing growth arrest, suggesting that an alternative kinase phosphorylates WTS-1/LATS redundantly with CST-1/2 in intestinal cells. Studies in Drosophila and mammalian cells indicate CNH-MAP4 Ste20 kinases can compensate for Hippo in activating Warts/LATS1/2. We hypothesized that MIG-15 and GCK-2, CNH-MAP4 Ste20 kinases in C. elegans, act redundantly with CST-1/2 to activate WTS-1/LATS and hence repress YAP-1. Conditional mig-15 depletion in cst-1/2 mutants induced L2 arrest, reversible by yap-1- or egl-44/TEAD-directed RNAi, thereby phenocopying loss of WTS-1/LATS. However, additional gck-2 deletion did not exacerbate this phenotype, leaving its role in the Hippo signaling network unclear. Our findings reveal a canonical Hippo pathway in the hypodermis and a non-canonical, MIG-15-mediated mechanism redundant with CST-1/2 in the intestine, offering new insights into Hippo signaling complexity.

321A Characterization of a new uterine eggshell layer in the nematode *C. elegans*

Mohamad Alkhatib, Sara K. Olson Biology, Pomona College

Metazoan oocytes are coated with a matrix of secreted extracellular glycoproteins known as the egg coat. Before fertilization, these proteins may play a role in sperm-egg recognition. After fertilization, they serve as a scaffold upon which the remainder of the egg coat will be built to generate an impenetrable barrier that protects the embryo during development. In nematodes, this early egg coat is called the Vitelline Layer, which is currently thought to form the first and outermost layer of the multilaminar eggshell. While characterizing the core vitelline layer proteins CBD-1, PERM-2, and PERM-4, we identified several protein interactors through co-IP/mass spec. CRISPR-Cas9 gene editing was used to generate fluorescently-tagged versions of these new proteins, as well as deletion mutants. Interestingly, these new proteins are not secreted by the newly fertilized embryo (as other eggshell proteins are), but are expressed in the spermatheca and/or uterus, suggesting that they contribute to an uncharacterized external eggshell layer that is coated on top of the existing eggshell structure. The existence of this external uterine layer has been theorized, but not studied. The new uterine layer proteins fail to assemble onto the outer eggshell in the absence of vitelline layer proteins, underscoring the intricate and hierarchical nature of nematode eggshell assembly. Interestingly, few of the uterine layer genes exhibit a loss-of-function phenotype, suggesting there may significant functional redundancy among this collection of orthologous genes. This hypothesis has been verified through creation of null alleles of multiple uterine layer genes in a single worm strain. The genetic tools we are building will allow us to probe our current working model that proposes the uterine layer proteins serve to fill in the gaps of a more porous vitelline layer to help reinforce the impenetrable nature of the post-fertilization eggshell.

322A An auxin-inducible degradation-based toolkit for tissue-specific protein degradation during *C. elegans* embryogenesis

Neha Varshney[1], Rebecca Green[2], Noura Yassir[1], Pablo Lara-Gonzalez[3], Arshad Desai[1], Karen Oegema[1]1UCSD, [2]Biology, UCSD, [3]UC Irvine

In the *C. elegans* embryo, proteins essential for cellular functions are often repurposed for distinct functions in differentiated cells during development. Studying these dual-function proteins is challenging because genetic knockouts and RNAi-based perturbations often cause early-stage defects, making it difficult to analyze later developmental roles. Additionally, determining a protein's function in specific tissues requires precise, tissue-targeted depletion. To address these challenges, we are developing a tissue-specific auxin-inducible degradation toolkit for *C. elegans* embryos. Auxin-mediated degradation relies on the plant F-box protein TIR1, which binds auxin and triggers proteasome-mediated degradation of target proteins tagged with an auxin-inducible degron (AID). Our system directs the expression of TIR1(F79G) using tissue-specific promoters, enabling targeted protein depletion in specific embryonic tissues. Since the expression of TIR1 from single-copy insertions can be insufficient for rapid degradation, we generated a set of heritable extrachromosomal arrays expressing TIR1 under the control of different tissue-specific promoters (intestine (*elt-2*), pharynx (*cest-17*), muscle (*hlh-1*), neuroblasts (*ztf-11*), neuron (*cnd-1*), and epidermis (*lin-26* or *elt-1*)). To facilitate tracking of array expression, TIR1 is expressed in an operon with mCherry::Histone H2B, and arrays are marked with the dominant marker *rol-6(su1006)* for easy strain maintenance. Efforts to target TIR1 to sub-tissue cell populations, such as seam cells *(ceh-16)*, and sensory neurons (*dyf-7*) are underway. To use these arrays, we introduce them into paired strains in which sequences encoding GFP alone (as a control) or GFP fused to an AID have been inserted into the endogenous locus of the target gene. The degradation efficiency of each array is also tested in a background expressing AID::GFP::MAD-1, a broadly expressed protein that localizes to the nuclear envelope but is not required for embryonic development. Using this system, we have identified tissue-specific phenotypes associated with the degradation of the kinetochore component BUB-1 and a PP1 phosphatase isoform, which will be described in other posters. In summary, the toolkit described here facilitates spatiotemporally controlled, tissue-specific protein degradation, allowing precise interrogation of cytoskeletal dynamics, morphogenesis, and other developmental processes in *C. elegans* embryos.

323B Toward cloning of genes regulating developmental speed

Kaho Ieda, Kotaro Kimura Grad Sch Sci, Nagoya City Univ

Development is the process by which a multicellular organism develops from a fertilized egg into an adult under spatial and temporal regulation. Temporal regulation of development is critical for ensuring the proper progression of development, yet it has been far less explored than spatial regulation, such as that mediated by segmentation genes. Developmental speed has been known to correlate with temperature in many animal species (Gillooly *et al.*, Nature 2002), although regulators coordinating the complex biochemical reactions have not been sufficiently elucidated. To address this, we are trying to reveal new genes regulating developmental speed in the nematode *C. elegans*, specifically the ones that accelerate the speed when mutated. We have conducted a forward genetic screen of approximately 20,000 haploid genomes to identify mutations accelerating developmental speed, isolated three mutant strains whose developmental speed increased significantly from that of wild-type animals, and mapped a causative gene candidate to 0.79 m.u. on chromosome V. In future, we will identify the causative genes and reveal the physiological function of the gene and genetic pathway to understand the evolutionarily conserved mechanisms of temporal regulation of development.

324B Full-length TRA-1 is a Gli activator that requires TRR-1 to function

Jibran Imtiaz, Youngquan Shen, Ronald Ellis Molecular Biology, Rowan-Virtua SOM

Gli transcription factors are involved in cell fate determination, proliferation, and patterning in many species and are major effectors of Hedgehog (Hh) signaling. There are three Gli proteins in humans, and mutations or errors in their regulation lead to a variety of developmental disorders or cancers. Most Gli proteins form full-length activators and cleaved repressors. Previously, the key nematode sex-determination gene, *tra-1,* had been thought to make only a Gli repressor.

By analyzing numerous mutations in a conserved domain of TRA-1 that is removed by cleavage when repressor is produced, we show that full-length TRA-1 promotes spermatogenesis but does not regulate somatic fates. This activator domain is required for full expression of *fog-1* and *fog-3,* which directly promote spermatogenesis. Furthermore, its activity is conserved between *C. briggsae* and *C. elegans.*

Mutations in TRR-1 behave similarly to TRA-1 activator mutations, and genetic epistasis suggests they the two genes act at the same point in the pathway. Thus, TRR-1 and the associated Tip60 HAT complex might work together with TRA-1 to promote transcription. To study potential co-factors like this, we have tagged TRA-1 with a 7x His-tag at amino acid 129. Both the *XX* and *XO* animals develop normally, so TRA-1 retains wildtype function. We are using this strain to purify TRA-1 produced in wildtype or mutant strains using nickel columns. We can use the protein for mass-spectrometry, to identify TRA-1 modifications, and learn if TRR-1 or other genes control these modifications.

To purify native TRA-1 complexes, we are making a similar 3X-FLAG tag insertion, so that we can directly identify co-factors by mass-spectrometry. Since worms lack classical Hedgehog signaling, study of nematode TRA-1 should reveal other types of Gli regulation. Furthermore, we suspect that regulation of TRA-1 activator played a major role in the evolution of hermaphrodite spermatogenesis in nematodes.

325B The role of neuropeptides in developmental progression and arrest

Erin Jackson[1], Benjamin Mulford[2], Victoria Jordan[1], Taha Mirrani[1], Pamela Najm[1], Bruce Wightman[11]Biology, Muhlenberg College, [2]Muhlenberg College

C. elegans nematodes can halt development in response to high salt conditions in a process called peri-hatching arrest. A synthetic peri-hatching arrest phenotype can be created by mutating the *daf-2/insulin receptor* along with the *fax-1* or *unc-42* transcription factors, both of which are involved in the differentiation of a subset of neurons. This indicates that development past the peri-hatching arrest phase depends on both insulin signaling and neuronal function. We are investigating the role of signaling molecules such as insulin peptides and neuropeptides in this developmental arrest pathway.

Nematodes have one insulin receptor (*daf-2*), but 40 different insulin eptides. We examined the role of various agonistic insulin peptides in the developmental pathway past the peri-hatching arrest phase. I created new mutant strains that combine different insulin peptide mutations with either *fax-1(gm83)* or *unc-42(e419)*. None displayed peri-hatching arrest. Candidate insulin peptides included *ins-3*, *ins-4*, *ins-5*, *ins-6*, and *daf-28*. These results could be due to redundancy in the function of various insulin peptides, physiological compensation, or because untested insulin peptides are responsible for signaling in this pathway.

We speculated that the nervous system component of peri-hatching arrest might depend on neuropeptide signaling, given the system-wide response. Consistent with this hypothesis, *egl-21; daf-2* double mutants also exhibit peri-hatching arrest. The *egl-21* gene encodes a carboxypeptidase crucial for processing neuropeptides. Candidate neuropeptides were chosen on the basis of expression in relevant neurons that also express the *fax-1* or *unc-42* transcription factors. In order to understand the specific contributions of neuronal signaling, we tested the salt sensitivity of neuropeptide mutants. Those mutants that displayed increased peri-hatching arrest in response to high salt conditions allowed us to identify candidate neuropeptides that may play a role in this arrest pathway. From here, we can create double mutant strains that combine the neuropeptide mutations with the *daf-2(e1370)* mutation. Increased peri-hatching arrest in these animals would suggest an essential role of neuropeptides in conjunction with insulin signaling in the development of *C. elegans* past the peri-hatching arrest phase.

326B The PP2A complex, the scaffold RACK-1, and 14-3-3 proteins interact with EXC-4/CLIC to regulate excretory canal outgrowth

Jordan Jesse, Anthony Arena, Daniel Shaye University of Illinois Chicago

G-protein coupled receptor (GPCR) and heterotrimeric G-protein (Gα/β/) pathways regulate many aspects of development and physiology, including vascular development and maintenance. Our recent studies demonstrate that Chloride Intracellular Channels (CLICs) are conserved players in Gα-Rho/Rac pathways. Prior work demonstrated CLIC1 and CLIC4 are required for angiogenic behaviors of human endothelial cells and for vascular development in mice. In human umbilical vein endothelial cells (HUVEC) two GPCR ligands, sphingosine-1-phosphate (S1P) and thrombin, induce transient CLIC1 and CLIC4 accumulation at the plasma membrane (PM), and these CLICs are differentially required to activate Rac1 and RhoA downstream of the S1P and thrombin receptors. The gene *exc-4* encodes a *C. elegans* CLIC that regulates excretory canal (ExCa) tubulogenesis. EXC-4 constitutively accumulates at the ExCa PM, and this accumulation is necessary for function. We used a reduced-function mutation, affecting a conserved motif in the EXC-4 C-terminal domain (CTD), to discover genetic interactions with *ced-10*/Rac and *mig-2*/RhoG mutants, demonstrating that *exc-4* function in Rho/Rac signaling is conserved. Moreover, we recently found that the CLIC1 and CLIC4 C-termini define their specificity in Rac1 activation, and that PM-localized CLIC4 CTD is necessary and sufficient for Rac1 activation in response to S1P. We hypothesize that EXC-4/CLIC function is mediated by physical interactions at the PM between conserved motifs in the CTD and regulators of Rho/Rac activity. To test this hypothesis, we immunoprecipitated (IP'd) fluorescently-tagged EXC-4 expressed in the ExCa and identified Co-IP'd proteins via mass-spectroscopy. We found the three subunits of PP2A (LET-92, PAA-1, PPTR-1), the scaffold RACK-1, and the 14-3-3 orthologs PAR-5 and FTT-2, all of which have been implicated in Rho/Rac signaling, as putative EXC-4 interactors. We are undertaking biochemical approaches to establish that these are *bona fide* physical interactors and genetic approaches to define their function in EXC-4/CLIC-mediated Rho/Rac signaling in both C. elegans and HUVEC.

327B Screening for Invasion Inhibitors: Insights from *In Vivo* and *In Vitro* Systems

Weichen Kao, Simon Berger, Alex Hajnal Department of Molecular Life Sciences, University of Zurich

Understanding cell invasion and identifying potential inhibitors are important in cancer treatment, preventing the formation of metastasis. *C. elegans* serves as an ideal model system for this purpose thanks to the regular occurrence of cell invasion events during natural development. We employed a novel microfluidic-based, high-throughput imaging approach, developed in our lab, to assess the effect of 1280 small molecule drug compounds (LOPAC library) on basement membrane breaching during *C. elegans* anchor cell invasion, and identified 14 compounds resulting in inhibited BM breaching. The identified compounds were validated in *C. elegans* as well as mammalian 2D and 3D cell culture systems, identifying several compounds with a potentially conserved inhibitory effect. Meanwhile, we tested compounds with similar targets and effects through the same process. We then identified five additional compounds from the same derivative group that exhibited a stronger phenotype. Many of which have previously been shown to inhibit tumor progression, but no common receptor target has been identified so far. In future experiments, we aim to understand whether these compounds act through the same mechanism in both *In vivo* and *In vitro* systems. We will select the three most potent compounds to identify genes of interest. Following that, RNAi knockdown will be applied to see if these genes are involved in the invasion process. In this way, we hope to not only broaden our understanding of cell invasion across different model systems but also identify several compounds with a possible therapeutic effect preventing the formation of cancer metastasis in humans.

328B Suppressor screens of ddx-15 helicase do not show interacting partners

Jonathan Karpel[1], Judith Yanowitz[21]Biology, Southern Utah Univ, [2]Magee-Womens Research Institute

Suppressor screens are useful to try to identify genes with some relationship or interaction with your gene of interest. Working with a helicase whose function is still not elucidated in *C. elegans*, we ran a suppressor screen and RNA-seq experiments on a helicase mutant in order to gain information on potential interacting proteins. The suppressor screen resulted in only a particular chromosomal rearrangement in the animals, but did not pinpoint any interacting proteins of interest. The RNA-seq experiment showed several genes with significant alterations to expression profiles, and these genes were targeted for RNA interference experiments in the mutant background. Results of the RNAi experiment will be forthcoming.

329B The AP-1 clathrin adaptor complex differentially regulates LIN-12/Notch signaling

Tatsuya Kato[1,2], Olga Skorobogata[1,2], Soroosh Siyoofi[1,2], Christian Rocheleau[1,2,31]Research Institute of the McGill University Health Centre, [2]Department of Anatomy & Cell Biology, McGill University, [3]Department of Medicine, McGill University

Notch signaling produces developmental outcomes dependent on the cellular context, thus it is important to understand its regulation in different tissues. *C. elegans* vulval development is a powerful model to this end since it is controlled by LIN-12/Notch-dependent cell fates in the gonad and epidermis. In the somatic gonad, LIN-12/Notch activity specifies the ventral uterine (VU) versus anchor cell (AC) fates, with VU receiving more signal than the AC. The AC secretes epidermal growth factor (EGF) which induces the underlying vulval precursor cells (VPCs) to adopt vulval fates. In the VPCs the secondary fates are specified by LIN-12/Notch activity. Aberrant Notch signaling causes vulval defects such as ectopic secondary inductions or lack of inductions called the Vulvaless phenotype. LIN-12/Notch activity is influenced by intracellular trafficking which is regulated by proteins such as adaptor protein complex 1 (AP-1), a conserved clathrin adaptor involved in polarized sorting via the trans-Golgi network. We have shown that AP-1 regulates EGF receptor (EGFR) signaling in the VPCs by antagonizing LET-23/EGFR basolateral localization with AP-1 recruiter ARF-1 and AGEF-1 ArfGEF. However, it is not known how AP-1 regulates Notch signaling during vulval development.

We analyzed a null allele *sy108* of the AP-1 medium subunit *unc-101/ap1m1* in a *lin-12(n302)* partial gain-of-function (GOF) Vulvaless background which inhibits AC specification but is insufficient for ectopic secondary induction. *unc-101(sy108)* suppressed the *lin-12(n302)* Vulvaless background through AC fate restoration, indicating that AP-1 promotes LIN-12/Notch signaling in the somatic gonad. Interestingly, these double mutants exhibited ectopic secondary inductions, suggesting that AP-1 inhibits LIN-12/Notch in the VPCs. Expressions of LIN-12/Notch receptor and LAG-2/DSL ligand endogenous reporters as well as a Notch signaling biosensor confirmed that AP-1 loss decreased LIN-12/Notch activity in somatic gonad cells while increasing it in the VPCs. ARF-1 RNAi knockdown and an *agef-1(vh4)* GOF allele led to largely similar suppressions of the Vulvaless phenotype, ectopic secondary inductions, and LIN-12/Notch signaling changes. Our results point to differential LIN-12/Notch signaling regulation by AP-1, likely through tissue-specific LIN-12/Notch sorting. We are currently analyzing PAR-6, LET-413, and LGL-1 localizations to determine if this differential regulation is additionally due to AP-1's maintenance of VPC polarity.

330B Novel regulatory interactions in *C. tropicalis* sex determination

James F Kennedy[1], Ronald Ellis[2], Soumi Joseph[21]Cell and Molecular Biology, Rowan-Virtua SOM, [2]Rowan-Virtua SOM

Hermaphrodite sex determination is shared between *C. elegans*, *C. briggsae*, and *C. tropicalis*, but this trait is thought to have evolved independently in each species. Genes of the core sex-determination pathway are largely conserved in the Caenorhabditis genus, including those encoding the FEM complex proteins: FEM-1, FEM-2, and FEM-3. These proteins were discovered in *C. elegans,* where they are necessary for both male sex determination and spermatogenesis, as shown by null mutants that completely feminize the soma and germline in *XX* and *XO* animals. Surprisingly, these phenotypes are not all shared in *C. briggsae* where *fem-2* and *fem-3* null mutants result in *XX* and *XO* animals becoming hermaphrodites. This difference suggests a divergence in the role of the FEM complex in these two species.

We made the mutant strain *cbr-fem-1(v508)* which consisted of fertile *XX* and *XO* hermaphrodites*,* an identical phenotype to *C. briggsae fem-2* and *fem-3* null mutants. To place *cbr-fem-1* in the sex determination pathway, double mutants were produced with the suspected upstream (*tra-2*) and downstream (t*ra-1*) genes. The results resembled those for *cbr-fem-2* and *cbr-fem-3,* with *tra-1(v181); fem-1(v508)* mutants making *XX* males and *tra-2(nm1)*; *fem-1(v508)* mutants making *XO* hermaphrodites. Finally, a *fem-2(nm27); fem-3(nm63) fem-1(v517)* triple mutant strain has an identical phenotype to that of the individual null mutants. We infer that the function of the three FEM proteins in a complex is heavily conserved in Caenorhabditis.

Although neither cbr-FEM-1, nor the other members of the complex are required for spermatogenesis, mutations in these genes weakly promote oogenesis in *tra-1* mutants. Thus, the *fem* genes have an unknown function downstream of *tra-1* in both *C. elegans* and *C. briggsae.*

For comparison, we made null alleles of *C. tropicalis* sex determination genes. The null mutants *tra-1(v547)* and *tra-2(v548)* make *XX* males, and *fem-1(v470)* makes *XX* and *XO* females. The *ctr-tra*-2 *XX* males make much better tails than in the other species. *C. tropicalis fem-1(v470)* mutants show germline feminization like in *C. elegans*, however, the double mutant *tra-1(547); fem-1(v470)* makes sperm. This result shows that the role of *fem-1* in the germ line is unique in each of the three hermaphroditic species.

331B Role of TXBP-3 in regulating spermathecal contractility through Rho Signaling in *C. elegans*

Maria Khalid, Avery Lord, Erin Cram Biology, Northeastern university

TXBP-3/TAX1BP3 is a small, highly conserved protein consisting of a single PDZ domain, which may interfere with interactions between PDZ-containing proteins. The human ortholog participates in various signaling pathways, impacting adhesion, migration, polarity, and cell proliferation. In *C. elegans*, TXBP-3 is expressed in multiple contractile tissues, including the pharynx, spermatheca, spermatheca-uterine valve, and rectal epithelium. We analyzed the role of TXBP-3 in the spermatheca, which is the site of fertilization in the hermaphrodite reproductive system. When *txbp-3* is deleted, the transit of oocytes through the spermatheca is delayed. Conversely, overexpression of TXBP-3::GFP leads to rapid transit of oocytes, and also excessive squeezing and pinching of oocytes which decreases fertility. Analysis with a genetically encoded Rho sensor suggests that TXBP-3 inhibits Rho activity. These results suggest that TXBP-3 regulates spermathecal contractility through Rho.

332B Reproductive consequences of developmental arrest and evolutionary changes in lab cultured *C. elegans*

Ashley Kim, Jocelyn Mertz, Bruce Wightman Muhlenberg College

C. elegans can arrest development at different stages. Perihatching arrest refers to a novel arrest phenotype that occurs between hatching and L1 in fax-1;daf-2 double mutants. Osmotic stress promotes perihatching arrest, while insulin signaling opposes it. We wanted to explore the potential metabolic or health implications of arrest. Brood size can be a proxy for overall nematode health and metabolism, so we measured the brood sizes of arrested worms. We found that arrested animals had brood sizes similar to controls, suggesting no reproductive cost associated with developmental arrest. Ongoing longevity studies will provide another measure of the potential cost or advantage of developmental arrest.

While conducting these studies, we discovered that our laboratory N2 strain showed a reproducible decrease in brood size. Our review of literature revealed inconsistent N2 brood sizes across labs, suggesting the possibility of evolutionary changes in populations cultured in laboratories over long periods of time. We are conducting various measures of strain health and phenotype to understand how continuous culture could decrease brood size, an apparent contradiction to expected selective pressure in favor of greater reproductive success.

333B unc-32 interferes with EMS spindle orientation and the cell cycle of the E-blastomere

Jiyu Kim, Olaf Bossinger Institute of Anatomy I, Molecular Cell Biology, University of Cologne

The unc-32 mutant was originally isolated by Sydney Brenner in 1974 and cloned by Pujol et al./Oka et al. in 2001. The unc-32 gene encodes the vacuolar (H+)-ATPase subunit *a* in C. elegans. V-ATPases are multi-subunit complexes that provide acidification of intracellular organelles essential for various cellular functions. We came across unc-32 in a small RNAi screen to identify genes that inhibit embryonic intestinal differentiation (no birefringence/autofluorescence). 4D microscopy of unc-32 embryos (n=102) revealed a slowing of the cell cycle (CC) in all somatic founder cells and the germline, but each lineage retained its identity with respect to the cell cycle length, with one exception: The CC of the E blastomere, the endodermal precursor cell, became almost as fast as that of its sister cell MS, the mesodermal precursor cell. CC acceleration in the E lineage of unc-32 embryos was strongly dependent on the spindle orientation in the EMS mother cell. In wild type, EMS divides asymmetrically along the anterior-posterior axis. In contrast, 60% of unc-32 embryos showed a left-right (l/r) or dorsal-ventral (d/v) spindle orientation in EMS, leading to an significant acceleration of the CC in the E lineage. In C. elegans MES-1/SRC-1 and Wnt signaling act together to control EMS spindle orientation (Bei et al., 2002; for review see Ishidate et al., 2013). Next, we performed double depletion of unc-32 with either MES-1/SRC-1 (n=39) or Wnt pathway components (n=17). In each experiment the EMS spindle was predominantly aligned along the lr/dv axis of the embryo (82%/88%, respectively). Thus, unc-32 significantly enhances the spindle phenotype of both, mes-1/src-1 and wnt single mutations. The unc-32 mRNA (HCR) and the UNC-32 protein (peptide antibody) are ubiquitously expressed in early embryonic stages and do not provide further evidence for its function. The C. elegans genome harbors 22 orthologs of 14 vacuolar (H+)-ATPase subunits. To date, depletion of these orthologs has not resulted in an unc-32-like phenotype, suggesting that unc-32 most likely acts independently of vacuolar (H+)-ATPase function.

334B Role of MAB-3 in robustness of morphogenesis

Karin Kiontke[1], Mary Loukaitis[2], Sophia Heimbrock[3], Raya Jallad[1], David H. A. Fitch[31]Biology, New York University, [2]The Bronx High School of Science, [3]New York University

Most developmental processes are robust to environmental and genetic perturbations. In *Caenorhabditis elegans*, vulva cell fate specification is a well-studied example of robust development [Felix & Barkoulas, 2012, Trends Genet 28:185-95]. Another example of robust development is Tail Tip Morphogenesis (TTM), the process by which the male tail tip changes shape from long and pointed to short and round during the L4 stage. Previous work showed that the DM-domain transcription factor DMD-3 is the master regulator of TTM [Mason et al, 2008, Development 135:2373-82] and occupies a central position in a bow-tie regulatory network [Nelson et al. 2011 PLoS Genet 7:e1002010]. In the absence of *dmd-3*, TTM fails and all adult males have long (Lep) tail tips. Slight TTM defects are also observed in mutants of the *dmd-3* paralog *mab-3*. We found that the *mab-3(-)* phenotype is temperature-sensitive and increases in frequency and expressivity with stressful temperatures. Thus, MAB-3 plays an important role in the robustness of TTM against thermal perturbations.

We are investigating the possible mechanisms by which MAB-3 contributes to this robustness by testing several hypotheses: (1) MAB-3 could act by maintaining the expression of *dmd-3* above a threshold level. That this might be true is indicated by single tail tip transcriptome profiling (see abstract by Raya Jallad et al.) showing reduced *dmd-3* transcript levels in *mab-3(-)* mutants compared to wild-type at 25°C. We are in the process of performing ChIP-seq for MAB-3 to determine if this interaction is direct. Alternatively: (2) MAB-3 could act on the same genes in the same way as DMD-3, or (3) MAB-3 could target another gene that conveys robustness to TTM, e.g. a heat-shock gene like *hsp-90*. Transcriptome analysis provides support for these hypotheses as well.

We also investigate other factors that may influence the robustness of TTM, e.g. salinity, pH, oxidative stress and passage through the dauer stage.

335B *pde-2* regulation by FBF proteins is crucial for brood size maintenance in *Caenorhabditis elegans*

Kamal Kishore, Jagannath J, Vandita Bhat, Inchara Muralidhara, Kavita Babu Centre for Neuroscience, Indian Institute of Science

Cell signaling is crucial for communication between cells and maintaining cellular homeostasis. PDE-2, a phosphodiesterase, regulates the intracellular levels of cyclic guanosine monophosphate(cGMP) and cyclic adenosine monophosphate (cAMP). These cyclic nucleotides are important secondary messengers that mediate multiple physiological processes, including chemotaxis behavior, body size regulation, and sensory transduction in *C. elegans*. Evidence has shown a possible interaction of *pde-2* RNA with the *fem-3* binding factors (FBF), RNA-binding proteins responsible for post-transcription regulation. Using yeast three-hybrid system, we have shown that FBF proteins bind to *pde-2* mRNA at its 3›UTR. To further validate this interaction and its effects, we mutated the FBF binding site within the *pde-2* 3› UTR using the CRISPR-Cas9 system. Our qPCR experiments show that the expression of *pde-2* increased significantly in these mutants. FBF proteins are expressed in the gonads of *C. elegans* and are known to regulate its germline development. Additionally, studies have also shown the presence of *pde-2* transcript in *C. elegans* gonads, indicating a possible interaction in gonads. Our data demonstrate a defect in the total brood size when the FBF binding site is disrupted. We also performed RNA sequencing in these mutants and showed that the genes involved in spermatogenesis are differentially regulated. We further plan to confirm the PDE-2 expression in gonads by knocking in a fluorescent protein at the *pde-2* genomic locus, which will allow us to study its spatiotemporal regulation by FBF proteins. Additionally, we intend to study the difference in the sperm pool in the mutants to confirm the role of *pde-2* in spermatogenesis, which in turn may provide a plausible explanation for maintenance of brood size.

336B The mitochondrial trans-2-enoyl-coA reductase is necessary for oogenesis in *C. elegans*

Peter Kropp[1], Katherine Spilsbury[2], Rosa Kwon[2], Elizabeth Manning[2], Ava Merickel[21]Biology, Kenyon College, [2]Kenyon College

The mitochondrial trans-2-enoyl CoA reductase (MECR) is the terminal enzyme of the mitochondrial fatty acid synthesis (mtFAS) pathway, an enigmatic pathway producing fatty acids that function as cofactors for mitochondrial enzymes. These fatty acids vary in length from eight to 16 carbons, and are essential for the function of multiple enzymes in cellular respiration. *MECR* is also the causative factor in MEPAN syndrome, a rare, autosomal-recessive disease characterized by dystonia, neurodegeneration and optic nerve atrophy. To better understand the role of *MECR* in mitochondrial and organismal function, we knocked out its ortholog, *mecr-1*, in *C. elegans*. Although the *mecr-1* null homozygotes have grossly normal development, they are sterile due to impaired gametogenesis. Hermaphrodites produce sperm, but fail to produce oocytes in a phenotype that does not replicate typical masculinization of the germline (*mog*) phenotypes. We have used immunofluorescent analyses of spermatogenic and oogenic regulators to determine whether this is a failure to terminate spermatogenesis, a failure to initiate oogenesis, or the result of broader germ cell dysfunction. We have found that spermatogenesis appears broadly normal with sperm expressing FOG-3 and MSP, but that there are precocious MSP+ cells in the distal gonad. Furthermore, the undifferentiated germ cells do not express LIN-41, a pro-oogenic factor, despite diplotene germ cells expressing this marker. These results suggest that there is an impairment in gametogenesis *writ large*, but that the progression through oogenesis is disproportionately affected. Ongoing work is investigating if the RNA-binding proteins that regulate gametogenesis are disrupted and dysfunctional which may lead to the mechanistic connection between MECR-1 and oogenesis.

337B An Ultrastructural Time Series Covering *C. elegans* Embryogenesis

Eric Krueger[1], Christopher K.E. Bleck[1], Grant Kroeschell[1], Molly Reilly[1], Mark Kittisopikul[1], Diyi Chen[1], Sarah Emerson[2], Anthony Santella[3], Zhirong Bao[3], Daniel Colon-Ramos[2], Hari Shroff[11]HHMI Janelia Research Campus, [2]Department of Neuroscience, Yale University School of Medicine, [3]Developmental Biology Program, Sloan Kettering Institute

The primary nerve tissue, or neuropil, in the nematode *Caenorhabditis elegans* is organized into distinct anatomical layers that correspond to specific behavioral circuits. However, the mechanisms underlying the development of this organizational structure during embryogenesis remain to be fully elucidated. To investigate this process and characterize the dynamics of neuronal growth and neuropil assembly over time, we utilize enhanced focused ion beam scanning electron microscopy (eFIB-SEM) to achieve high-resolution, isotropic imaging of entire *C. elegans* embryos across diverse developmental stages. On this poster, we detail our advancements in performing comprehensive body-wide segmentations at the resolution of individual cells, organelles, and larger organ systems.

338B Context-dependent Ligand Activity of TGF-β signaling in Innate Immunity of *C. elegans*

VenkataKrishna Lappasi Mohanram, Emma Jo Ciccarelli, Zachary Wing, Cathy Savage-Dunn Biology, Queens College

The TGF-β superfamily regulates a broad spectrum of biological processes, including innate immunity by modulating host responses to pathogens. While the BMP ligand DBL-1 has a well-established role in *C. elegans* immunity, the contributions of other TGF-β ligands remain unclear. Here, we investigate the roles of TIG-2 (BMP-like), TIG-3 (TGFβ/Activin-like), and the Type II receptor DAF-4 in innate immunity, focusing on their interactions and regulatory mechanisms. Survival assays using *P. luminescens* revealed that mutants for all five *C. elegans* TGF-β ligands had reduced survival, with *tig-2* and *tig-3* mutants showing the most severe defects. Double mutants (*tig-2; tig-3*) exhibited survival rates similar to the single mutants, suggesting a nonredundant function. Further analysis under *P. aeruginosa* infection confirmed these findings, with *tig-2* and *tig-3* mutants showing significantly lower survival than wild-type (N2) animals. Surprisingly, *daf-4* mutants did not show a significant reduction in survival, suggesting that TIG-2::TIG-3 may signal in the absence of a Type II receptor. To explore the molecular basis of TIG-2 and TIG-3 cooperation, we performed structural modeling using ColabFold, which supports the formation of TIG-2:TIG-3 heterodimers with a high interface-predicted template modeling (ipTM) score (0.803). In contrast, TIG-3 homodimers were poorly supported (ipTM: 0.246). To validate these interactions *in vivo*, we will employ CRISPR/Cas9 knock-in technology to introduce epitope tags and flexible linkers into the prodomain and mature domains of TIG-2 and TIG-3. These modifications will enable us to assess ligand interactions before and after pathogen exposure, providing direct evidence for the formation of TIG-2:TIG-3 heterodimers in response to infection. Direct evidence of non-redundant, cross-family interactions between BMP and TGF-β/Activin ligands in immune regulation will be a novel contribution to the understanding of the context-dependent roles of TGF-β ligands. This approach will also help distinguish their developmental roles from acute immune functions, providing key insights into how TGF-β signaling fine-tunes host defense mechanisms.

339B Cyclin B3 coordinates cell division with cell fate specification during *C. elegans* early embryogenesis

David Bojorquez, Gio Jison, Pablo Lara-Gonzalez University of California Irvine

Embryonic development requires the coordination of mitotic division with cell differentiation to give rise to a fully formed organism. The Cdk1-cyclin B complex promotes mitosis by phosphorylating thousands of cellular substrates. Prior work from our lab showed that an isoform of cyclin B, known as cyclin B3 (CYB-3), is the main driver of early cell divisions in *C. elegans* embryos and it acts to accelerate the pace of mitoses. In our efforts at structure-function analysis of CYB-3, we identified a conserved phosphate binding surface on the surface of the protein. A similar patch was found previously in vertebrates and it was shown to bind to phosphorylated Cdk1 substrates to drive their hyperphosphorylation. We found that mutating this surface (PBSMut) did not result in major cell division defects nor disruption of the CDK-1–CYB-3 complex. However, CYB-3 PBSMut caused a fully penetrant embryonic lethality phenotype. Therefore, the CYB-3 PBSMut is a separation-of-function mutant that allows us to study the specific contributions of the CDK-1–CYB-3 complex in development. We next characterized the developmental phenotypes arising from CYB-3 PBSMut embryos and we found that, while early mitotic divisions proceeded normally, mutant embryos had severe defects in the specification of their germ layers and lost their ability to undergo morphogenesis. Among the putative molecular targets of the CDK-1–CYB-3 complex were the RNA binding OMA proteins (OMA-1 and OMA-2). OMA proteins are expressed in mature oocytes, where they act as both transcriptional and translational repressors. The degradation of OMA proteins during the first zygotic division is critical for the expression of early cell fate specification markers, thereby initiating the developmental program. We found that, whereas embryos expressing wild-type cyclin B3 degraded OMA before the onset of the first mitotic division, embryos expressing PBSMut cyclin B3 did not. However, inducing ectopic degradation of OMA through the auxin-induced degron (AID) system did not suppress the embryonic lethality of CYB-3 PBSMut, suggesting that the CYB-3 PBS regulates other targets, in addition to OMA, to ensure normal embryogenesis. Based on these observations, we propose that the CDK-1–CYB-3 complex orchestrates the early embryonic developmental program by (1) accelerating the pace of embryonic divisions and (2) regulating OMA and other to ensure proper cell fate specification. We are currently employing proteomics to identify interactors of the cyclin B3 phosphate binding surface during early embryonic development. Overall, these efforts will illuminate our understanding of how cell division and cell fate specification are coupled during embryonic development.

340B Stress prevents sex-specific muscle remodeling in males lacking ATFS-1

Brigitte LeBoeuf[1], Rene Garcia[21]Biology, Texas A&M Univ, [2]Biology, Texas A&M University

Male *C. elegans* remodel their anal depressor muscles during development to switch the muscle from defecation control to assisting in sperm transfer during mating. This switch is dependent on two different types of WNT signaling pathways, a cell-intrinsic calcium-dependent pathway that signals through egl-8/PLC-β, and a cell-extrinsic canonical pathway that signals through β-catenin in the surrounding epithelium. Metabolic changes accompany this remodeling, both to provide energy for the process itself, and meet the energy demands of the new muscle. The stress-response transcription factor ATFS-1 is involved in metabolic remodeling when the mitochondria membrane potential is reduced due to lower ATP production. Under non-stress conditions, ATFS-1 translocates to the mitochondria and is degraded. Mitochondrial stress prevents ATFS-1 from entering the mitochondria, sending the transcription factor to the nucleus to active metabolic pathways, including those involved in glycolysis. In males, ATFS-1 is sex-specifically up-regulated in the anal depressor during the L4 larval stage, when the existing dorsal/ventral sarcomere is disassembled and a new anterior/posterior sarcomere is created, suggesting it plays a role in the remodeling process. While the *atfs-1(lf)* males display occasional remodeling defects, the penetrance is greatly increased through the process of bleaching adult hermaphrodites and scoring their progeny. The reason exposing the eggs to bleach increases a developmental phenotype is unknown, but could possibly be due to stripping a coating off of the eggs, as the phenotype increase can be seen just through cutting the hermaphrodites open and scoring their progeny, albeit at a lower penetrance rate. Current work is focused on determining how early stress that impacts the eggs leads to remodeling defects, how ATFS-1 interacts with the WNT pathways, and where ATFS-1 is functioning to regulate muscle remodeling.

341B Dissecting the role of dhhc-8, a palmitoyl transferase, in fertility in *C. elegans*

Katelyn Edelbach, Katherine Maniates, Aastha Lele, Saai Suryanarayanan, Andrew Singson Waksman Institute of Microbiology, Rutgers University

There are 15 predicted DHHC genes identified in C. elegans. Previous work has revealed that two other palmitoyl transferases spe-10 and spe-21 were both required for post meiotic sperm differentiation, also known as spermiogenesis or sperm activation. Analysis of the structure of both the spe-10 and spe-21 genes revealed a Palmitoyltransferase Conserved C-Terminus (PaCCT) domain in these proteins. The PaCCT domain is characterized by a varied 16 amino acid sequence with both polar and basic amino acids. This domain is highly variable but is predicted to provide specificity to certain sets of substrates and localize to specific tissues. Analysis of the other predicted DHHC genes identified one other gene, dhhc-8, also had a PaCCT domain. We wanted to determine if this domain was required for sperm specific expression and function in C. elegans.

Using the recently generated dhhc-8(ve568) null mutation, we saw that the mutants had morphologically normal germlines however they produced significantly reduced brood size. Further analysis revealed that while there is a weak embryonic lethality effect (20% of wild-type levels), this is not sufficient for the more than 90% decrease in brood size. We examined dhhc-8 mutant males and saw that they were unable to produce any progeny despite producing normal numbers of sperm. These phenotypes are consistent with the expression of dhhc-8 that we see in the membranes of the germline and sperm. Ongoing work includes assessing the ability of dhhc-8 mutant sperm to activate and ascertaining any epistatic effects with spe-10 and spe-21. This work helps to better understand how palmitoyl transferases convey specificity to certain tissues and to further define sperm activation.

342B MIG-21 is a novel regulator of Wnt and Netrin signaling in gonad migration identified from published scRNA-seq data and functionally validated in *C. elegans*

Xin Li[1], Kacy Gordon[21]Biology, University of North Carolina, Chapel Hill, [2]University of North Carolina, Chapel Hill

The gonad of *Caenorhabditis elegans* hermaphrodites is a longstanding model of cell migration, stem cell niche function, and organogenesis, but it has not yet been investigated using single-cell RNA-sequencing (scRNA-seq). Using a recently published scRNA-seq dataset of adult *C. elegans* hermaphrodites, we identified a previously unknown regulator of the leader cell of gonad migration (the distal tip cell, or DTC). The gene *mig-21* has the highest "marker score"–yet no known role–in the DTC. However, *mig-21* regulates cell migration in other developmental contexts. Using classical genetics techniques, RNAi knockdown, and live cell imaging, we discovered that *mig-21* acts synergistically with the Wnt and Netrin pathways to guide anteroposterior and dorsoventral phases of DTC migration at the level of signaling, not DTC cell structure. Known interactors of *mig-21* in other cell types–like PTP-3/Lar and DPY-19–also act with MIG-21 in DTC migration. Despite its expression in stationary adult DTCs, *mig-21* does not play a role in the cessation of DTC migration but instead seems to impart continued sensitivity of the DTC to Wnt and Netrin in adulthood. This study reveals additional complexity of signaling integration between major regulators of germline stem cell niche migration, and as a proof of concept it demonstrates the utility of scRNA-seq datasets in revealing testable hypotheses about genetic networks that were masked by redundancy in traditional screening methods.

343B Temporal Analysis of Embryonic Epidermal Morphogenesis in *Caenorhabditis elegans*

Fangzheng LI[1], Peiyue LI[2], Mao Onishi[1], Law King Chuen[1], Yukihiko Kubota[1], Masahiro Ito[11]Ritsumeikan University, [2]Japan Advanced Institute of Science and Technology

Caenorhabditis elegans epidermal morphogenesis is a four-dimensional process in which embryos progress through distinct developmental stages before hatching. Previous research on epidermal morphogenesis has largely relied on RNA interference (RNAi) to knock down key developmental genes to study their effects on embryonic development. However, a challenge with using RNAi is that, while the knockdown of these genes may result in embryonic developmental delay, visible phenotypic defects are often difficult to observe under microscopy. As a result, it is challenging to determine at which specific developmental stage the embryos begin to exhibit these defects. This highlights the need to incorporate the fourth dimension—developmental timing—to complement traditional approaches.

To address this problem, we developed a deep learning-based image analysis approach using time-lapse differential interference contrast (DIC) microscopy images to predict the embryonic stage (dorsal intercalation, ventral enclosure, rotation, 1.5-fold, and 2-fold) of RNAi-treated animals during embryonic epidermal development. To check whether our approach could be applicable to analyze gene function in epidermal development, we selected three genes —*leo-1*, *ajm-1*, and *tes-1*, each of which affects embryonic development at different stages—and applied RNAi treatment. By comparing the time required for each developmental stage, we successfully identified stage-specific developmental impacts from a temporal perspective for all three genes. Our results show that *leo-1* regulates the early stages of epidermal development, while both *ajm-1* and *tes-1* regulate the later stages. Incorporating the temporal perspective into the analysis of epidermal morphogenesis offers a novel way to understand the dynamics of this process and provides new insights into gene function during the developmental process.

344B The ubiquitin-ligase HECD-1 counteracts conversion of germ cells to neurons

Qinming Li[1], Baris Tursun[2], miram hoops[21]Institute of Cell and Systems Biology of Animals, University of Hamburg, Germany, [2]Molecular Cell Biology Unit, Institute of Cell and Systems Biology of Animals, University of Hamburg, Germany

A balanced state of the proteome by proteostasis (or homeostasis) is essential for cell function, development, and organismal viability. Dysfunctional proteostasis is a hallmark of aging and contributes to the onset of age-related diseases. Furthermore, studies have demonstrated that proteasome activity is required to maintain pluripotency in embryonic cells and during reprogramming to induced stem cells. Yet, specific functions of proteostasis factors during cellular reprogramming are not fully understood, especially in vivo.

To investigate proteostasis dynamics during transcription factor (TF)-mediated direct reprogramming (DR), we use the zinc-finger TF CHE-1, which specifies the glutamatergic ASE neuron fate in *C. elegans*. By performing ectopic expression of CHE-1 and RNAi screening for DR barrier genes, we identified the ubiquitin E3 ligase HECD-1 as a safeguard of germ cell to ASE neuron conversion. Depletion of *hecd-1* via RNAi or by CRISPR-mediated mutation consistently induces ASE neuron fate in germ cells with corresponding morphological changes to neuron-like cells. Additionally, smFISH-based assessment of endogenous gene expression confirmed the faithful conversion of germ cells to ASE neuron-like cells.

As part of the Ubiquitin-Proteasome System (UPS), HECD-1 promotes the degradation of proteins. However, the specific target proteins of HECD-1 are not well known. Therefore, we combined mass spectrometry of *hecd-1* mutants to detect changes in the proteome with RNAi to screen for corresponding target genes that suppress DR in *hecd-1* mutant animals. We identified candidate targets that show upregulated protein levels in *hecd-1* mutants and suppression of DR upon their depletion by RNAi: *lmn-1*, *hsp-90*, *cox-4*, *lsm-7,* and *dpf-6*. DR suppression does not result from eliminating or harming germ cells as the germline appears healthy in animals with double depletions of *hecd-1* and the newly identified targets, which was assessed using germ cell reporters such as *sun-1::mRuby*.

Our results indicate a network of proteins that promote DR upon loss of a proteostasis regulator such as HECD-1, thereby contributing to delineating the context of the UPS in TF-induced cellular reprogramming.

345B Investigation of dauer entry genes and role of phase separation during dauer molt

Daisy S Lim[1], Taeseung Lee[2], Jeong-Mo Choi[2], Junho Lee[31]The Research Institute of Basic Sciences, Seoul National University, [2]Department of Chemistry, Pusan National University, [3]Departement of Biological Sciences, Seoul National University

In harsh environments, the nematode *C. elegans* develops into dauer, a diapause stage specialized for long-term survival. Previous studies in dauer development have identified various signaling components that affect the decision to develop into dauer. However, the genetic factors that establish physiological characteristics of dauer larvae, such as presence of alae and radially constricted body, are not well-known. In this study, we analyzed transcriptome data from previous studies to identify dauer entry-specific genes. Genes that peak in expression at dauer entry (Lee et al., 2017) were filtered against the genes that are known to oscillate during four molting phases in non-dauer growth in reproductive cycle (Meeuse et al., 2020). This revealed 240 genes that are expressed specifically during dauer entry. Among these genes is *daf-42*, an essential gene for dauer development that encodes secreted proteins that are predicted to undergo phase separation. Further investigation of oscillating and dauer entry-specific genes during dauer molting will offer deeper insights into our understanding of dauer development.

346B HSP90 co-chaperones promote GLP-1/Notch signaling during germline and embryonic development in *C. elegans*

James Lissemore[1], Amanda Pancake[1], Olivia Zucaro[1], Jonathan Bastick[1], Vladimir Tchepak[1], MacKenzie Lee[1], Joshua Krach[1], Abigail Beer[1], Javier Lopez Rivera[1], Eleanor Maine[21]Biology, John Carroll University, [2]Syracuse University

The GLP-1/Notch signaling pathway is required for germline stem cell proliferation and early embryonic development in *C. elegans*. We have previously demonstrated an essential role for the molecular chaperone HSP90 (encoded by the *hsp-90* gene) in GLP-1/Notch signaling. The HSP90 chaperone system involves numerous co-chaperones with a variety of functions. To investigate whether HSP90 co-chaperones are also required for GLP-1/Notch signaling, we conducted a systematic RNAi knockdown by feeding of 17 predicted *C. elegans* co-chaperone orthologs in both wildtype N2 and sensitized *glp-1(bn18ts)* genetic backgrounds. As proxies for GLP-1/Notch signaling in the germline and in embryos, we measured brood sizes and embryonic and larval lethality and defects. The results fell into four categories:

1) no effect of co-chaperone knockdown on GLP-1/Notch signaling, e.g. *aipr-1*;

2) reduction in brood size only in *glp-1(bn18ts)*, e.g. *Y22D7AL.9*;

3) reduced brood size and increased embryonic/larval lethality or defect only in *glp-1(bn18ts)*, e.g. *ttc-1*;

4) reduced brood size and increased embryonic/larval lethality or defect in both N2 and *glp-1(bn18ts)*, e.g. *cdc-37*.

We conclude that a subset of HSP90 co-chaperones in *C. elegans* promote GLP-1/Notch signaling during germline and embryonic development.

347B The Secreted Protein SPE-36 is Dependent on Multiple Proteins for Proper Localization in *C. elegans* Sperm

A'Maya Looper, Amber Krauchunas Biological Science, University of Delaware

Fertilization is the process by which sperm and egg fuse to give rise to a zygote. Defects in the fertilization process can result in sterility. Studying the gamete molecular components and their interactions in *C. elegans* can help form hypotheses for potential models of human fertilization. Understanding the molecular aspects of fertilization is crucial due to its significant implications for fertility treatments and human health.

We use the model organism *C. elegans* to study a sperm-specific fertilization defective mutant known as *spe-36*. SPE-36 belongs to a group of 11 sperm-specific proteins required for fertilization. The loss of any one of these proteins renders the worm sterile but has no other impact on the organism. These mutant sperm are morphologically identical to WT sperm but they are unable to fertilize an egg. We propose that the interactions between the sperm proteins and the oocyte proteins function collectively, forming a dynamic interaction parallel to a synapse- referred to as the fertilization synapse.

SPE-36 is a secreted protein that localizes to the cell body and pseudopod of active sperm (spermatozoa). Due to the secretory nature of SPE-36 and its ability to remain bound to the pseudopod of the sperm, we hypothesize that it requires a binding partner(s) to keep it associated with the sperm. We set out to identify a SPE-36 binding partner by testing if SPE-36 localization depends on other sperm-specific fertilization proteins. To test this, I crossed SPE-36::GFP into 10 different *spe* mutant backgrounds. In a *spe-9, spe-38, spe-13, spe-42, spe-49,* and *spe-45* mutant background, SPE-36 is present but fails to localize to the pseudopod properly. Indicating that SPE-36 localization is dependent on these proteins. In contrast, SPE-36 localization is not dependent on *fer-14, spe-51*, or *spe-41*. Strikingly, we do not observe SPE-36 in *spe-40* mutant spermatozoa, which may suggest that SPE-40 may be the binding partner for SPE-36. We have refined our hypothesis and now propose that SPE-36 is part of a larger complex of sperm proteins, where it requires both direct and indirect interactions for its proper localization. We seek to identify which proteins directly or indirectly bind to SPE-36, and ultimately identify novel proteins that may also be part of this complex using mass spectrometry and *in silico* methods.

348B Systematic perturbation of NOTCH signaling activity and its application in drug discovery

Xin Luo, Simon Berger, Alex Hajnal University of Zurich

Notch signaling is an evolutionarily conserved pathway that serves as an essential regulator of cell fate determination, differentiation, and apoptosis in all animals. The Notch signaling pathway acts as a binary cell-fate switch and functions as an oncogene or tumor suppressor in different types of human cancer. Deregulation of the Notch signaling pathway affects the signaling networks that govern oncogenesis, tumor progression, invasion, and metastasis. Therefore, a comprehensive understanding of the regulatory mechanisms is crucial for designing novel drug compounds and therapies targeting Notch signaling.

We investigate the induction of the vulval precursor cells (VPCs) in *C. elegans* as an *in vivo* model to study the regulation of Notch signaling. By utilizing existing biosensors to detect Notch signaling activity in the VPCs (SALSA, Shaffer and Greenwald 2022), combined with our automated microfluidic-based screening platform (Berger et al. [unpublished]), we can systematically perturb and quantify changes in Notch signaling activity.

Using these methodologies, we conduct RNAi and small-molecule compound screens to identify genes regulating Notch signaling and lead compounds for developing pharmacological Notch pathway inhibitors. Finally, we will expand this screening strategy to include RAS/MAPK and WNT pathway biosensors to characterize the entire signaling network active during VPC induction.

349B A redundant GATA factor network specifies gut in *Pristionchus pacificus*

Melissa Guthrie, Charles Warden, Gina Broitman-Maduro, Morris Maduro Molecular, Cell and Systems Biology, University of California, Riverside

The gut precursor, E, is specified in *C. elegans* by the maternal factors SKN-1 and POP-1, through a cascade of GATA factors, MED-1,2 --> END-1,3 --> ELT-2,7. The MED/END/ELT-7 factors are present only in the Elegans Supergroup. *C. angaria* and other species outside this group appear to use the ELT-3 GATA factor to specify gut, in a simpler network of POP-1 --> ELT-3 --> ELT-2. The results suggest that the ancestral E specification factor was ELT-3, and that *elt-3* underwent a radiation at the base of the Elegans Supergroup. This rewiring of the network is an excellent example of Developmental System Drift. We have extended our studies of gut specification into *Pristionchus*. The ELT-3 family of GATA factors has undergone a radiation within the genus, with *P. pacificus* and its close relatives having seven ELT-3-like factors, and other species having fewer of these. Using smiFISH, we found that three of these in *P. pacificus* are closely linked and are expressed in overlapping patterns in the early E lineage, suggesting they act in gut specification redundantly. We used CRISPR/Cas9 to make mutations in *Ppa-elt-3.1*, *Ppa-elt-3.3*, and *Ppa-elt-3.5*. The single mutants have no phenotype, but double mutants show variably penetrant gutless phenotypes. We hypothesize that these factors participate in a gut specification gene network upstream of *Ppa-elt-2.* We are further testing the ability of these derived *Ppa-elt-3.x* factors to specify gut when introduced into *C. elegans* as transgenes. Our results suggest that an independent GATA factor radiation occurred in *Pristionchus* gut specification, with unexpected similarity to the radiation that occurred in *Caenorhabditis*, and that some aspects of gut specification have evolved convergently in the parallel expansion of these networks in the two genera.

350B Development of a Deep Learning-Based System for Cell Classification to Elucidate Cell Fate Mechanisms in Early *C. elegans* Embryos

Isseki Maeda[1,2], Koji Kyoda[2], Masahiro Ito[3], Shuichi Onami[2,31]Bioinformatics, Ritsumeikan University, [2]RIKEN Center for Biosystems Dynamics, [3]Bioinformatics, Graduate School of Life Sciences, Ritsumeikan University

A central goal in developmental biology is to understand the state of individual cells during embryogenesis to elucidate the mechanisms of fate determination. The characterization of cell states and fate determination mechanisms has been advanced through approaches utilizing genetic markers, allowing for the identification of cell states and the genes involved in fate specification. However, the number of genetic markers that can be simultaneously used is inherently limited, and a definitive technique for precisely determining the fate of individual cells has yet to be established. In this study, we developed a deep learning-based system to predict cell fates solely from histone GFP fluorescence images in the nematode *C. elegans* during early embryogenesis. We assume the chromatin structure corresponding to the cell state is reflected in the image of the cell nucleus, and this system recognizes differences in the structure. We developed a ResNet-based multi-class classifier trained on 3D nucleus images annotated with cell names. Applied to 12 cells from the 4-cell to 8-cell stages, our system achieved an overall accuracy of 0.5, roughly six times higher than that of a random classifier (0.083). We computed accuracy as the ratio of correctly classified images to 1,379 images in our validation set. Notably, while the accuracy for P1 lineage was 0.61, the accuracy for the AB lineage was only 0.37. This lower accuracy in the AB lineage was primarily due to confusion among sister cells, whereas cells derived from different mother cells were distinguished. These results are consistent with previous findings that in the AB lineage, symmetric division generates equivalent daughter cells, while in the P1 lineage, asymmetric division generates the founder cells each of which produces differentiated descendants. Currently, we are improving classification accuracy and analyzing misclassification patterns to reveal biological insights into cell fate determination. Additionally, we plan to apply our system to *pop-1* and *glp-1* mutants, which are known to exhibit cell fate alteration, to validate whether the alteration can be detected. This approach is expected to contribute to generating new hypotheses on changes in cell state associated with cell fate alterations in mutants and advance our understanding of developmental mechanisms.

351B Deciphering the pathways that maintain mitochondrial uniparental maternal inheritance

Valentine Melin, Justine Cailloce, Fanny Husson, Jorge Merlet, Vincent Galy Development, Adaptation and Aging (Dev2A), Sorbonne-Université, CNRS, INSERM

Mitochondria are essential intracellular organelles in eukaryotic cells that enable multiple biological functions, including energy production. They contain their own genome (the mtDNA) which represents only a tiny fraction of the cell's genes, but is nonetheless vital. In many species, sexual reproduction is based on the equal inheritance of both parental nuclear genomes and on the uniparental maternal inheritance of mitochondria and their mtDNA. The uniparental transmission of maternal mitochondria is achieved by the active and specific disposal of the sperm mitochondria and their mtDNA around fertilization.

In *C. elegans*, sperm mitochondria enter the embryo and are quickly and actively targeted by allophagy which is a specific autophagy pathway. Several proteins involved in sperm-derived mitochondria clearance have been identified in *C.* elegans. Among them there is the worm specific allophagy receptor ALLO-1 and three conserved proteins: the endonuclease CPS-6, FNDC-1 and PHB-2 which are respectively involved in the degradation of mtDNA and two mitophagy receptors. However, it was not clear how these factors interact together and whether they account or not for the complete degradation of sperm-derived mitochondria.

In order to clarify the genetic interaction and respective contributions of these factors, we conducted a systematic and quantitative functional characterization of the different mutants. We also monitored if their simultaneous inactivation could prevent the clearance of sperm mitochondria and results or not in a biparental mtDNA inheritance.

I will present our results and how they reveal that at least two parallel pathways exist to ensure the elimination of sperm mitochondria in order to maintain a strict uniparental mitochondria inheritance.

352B Uncovering the function of XOL-1, the binary switch protein governing sex determination and dosage compensation in *Caenorhabditis elegans*.

Hector Mendoza, Alyssa R Sun, Camila Quintero Diaz, Anati A Azhar, Györgyi Csankovszki MCDB, University of Michigan

Diploidy is the predominant strategy in sexually reproducing eukaryotes through which equal gene copy numbers are contributed by each parent. Sex chromosomes represent an exception to this rule, in which the genetic contributions of the sperm and the oocyte are different. Ultimately, this difference contributes to sex determination. Dosage compensation has emerged as a mechanism that equilibrates sex-linked expression during development. In *Caenorhabditis elegans*, genetic balance between the biological sexes is achieved by the downregulation of the two hermaphrodite X chromosomes via condensin-mediated chromosome compaction and chromatin modifications. This process is controlled by the master regulatory switch protein XOL-1, expressed exclusively in males, which triggers male development and inhibits dosage compensation to prevent silencing of the single male X chromosome. Despite its crucial role, the mode of action of XOL-1 is unclear, as its function remains largely unexplored since its biochemical characterization over two decades ago. Our goal is to study XOL-1 to define the mechanism regulating dosage compensation and sex determination. We will present two angles of exploration to deduce the biochemical function of XOL-1: (1) identification of its key interactors via co-immunoprecipitation assays and (2) examination of SDC-2, a downstream target crucial for the establishment of dosage compensation during the hermaphrodite developmental program. Together, these objectives will contribute to the characterization of the molecular mechanism through which XOL-1 operates and how it contributes to its ability to impact both sex determination and dosage compensation.

353B Class I histone deacetylase HDA-3 is involved in the regulation of oogenesis in *Caenorhabditis elegans*

Tomoki Mimura, Soma Tani, Shotaro Yamanaka, Masahiro Ito, Yukihiko Kubota Bioinformatics, Graduate School of Life Sciences, Ritsumeikan University

Oocyte maturation and ovulation are essential processes for oogenesis and fertilization in multicellular organisms. It has been shown that oocyte maturation is supported by signaling molecules secreted outside the oocyte. In this process, major sperm protein (MSP) secreted by sperms and contraction of somatic sheath cells are involved (McCarter et al., 1999; Miller et al., 2002). However, the underlying mechanisms of oogenetic cycle (spatial and temporal regulation of oocyte maturation) are not yet fully understood. Histone deacetylases (HDACs) are negative regulators of transcription that inhibit the binding of transcription factors to DNA, and their expression levels are epigenetically influenced by environmental factors (Hua et al., 2024). Among these molecules, it has been shown that class I HDACs are expressed in the gonads. However, their roles in germ cell differentiation and maturation have not yet been elucidated.

In this study, we conducted a comprehensive analysis of class I HDACs to investigate their reproductive functions. We found that both *hda-3* deletion mutant *hda-3(ok1991)* and missense mutant *hda-3(ix261)* exhibited a prolonged egg-laying cycle phenotype. Upon comparing the progeny count every 24 h starting from the L4 stage, *hda-3* mutants showed a lower progeny count compared to the wild-type (WT) during the first 24 h (Day 1). WT exhibited a peak in progeny count on Day 2, whereas *hda-3* mutants showed a peak on Day 3. In addition, *hda-3* mutant continued to lay eggs after Day 4, whereas only a few eggs were laid by WT. However, when we measured the total number of lifetime offspring, the number was not significantly different between WT and *hda-3* mutants. Collectively, these results suggest that *hda-3* contributes to the promotion of oogenesis. To investigate the cell-type-specific functions of *hda-3*, we aim to use the germ cell-specific feeding RNA interference (RNAi) strain, DCL569: *mkcSi13 [sun-1p::rde-1::sun-1 3›UTR + unc-119(+)]* (Zou et al., 2019), and newly created sheath cell-specific feeding RNAi strain, KUB391: *rde-1(ne300); bkcSi63[lim-7p::rde-1::tbb-2 3'UTR, NeoR]*. Analyses of HDA-3-dependent oogenesis within gonads are expected to provide new insights into the mechanisms of oocyte maturation and ovulation.

354B Serine/Threonine Phosphatase SPE-57 is Involved in the Genetic Regulation of Sperm Activation

Emily K Mincher[1], Allison Ramz[1], Noah Dickison[1], Benjamin Khaim[1], Sarah Nicaj[1], Zain Uddin[1], Xue Mei[21]St. John's University, [2]Biology, St. John's University

Fertilization is a conserved process in sexual reproduction that depends on properly developed and differentiated gametes. In *Caenorhabditis elegans*, sperm activation is a post-meiotic differentiation process in which round, nonmotile spermatids form a motile pseudopod and become fertilization competent. During activation, Golgi-derived membranous organelles (MOs) fuse to the plasma membrane of wildtype sperm. The sperm-specific cytoskeleton Major Sperm Protein (MSP) polymerizes during activation to allow for the formation and movement of the pseudopod. It is thought that two semi-redundant pathways regulate sperm activation in *C. elegans*. Hermaphrodites opt for the SPE-8 pathway, while males can use either the SPE-8 or TRY-5 pathway. Many regulators in these pathways are yet to be identified, leaving molecular mechanisms unclear. The temperature sensitive mutant *as47* was isolated in a forward genetic screen aimed at discovering fertilization molecules. Using whole genome sequencing (WGS) and transgenic rescue, we cloned the gene and identified *R03D7.8*, now named *spe-57*, as the affected gene in *as47.* Here, we characterize the null allele, *spe-57(tm5344).* Hermaphrodites are subfertile, lay unfertilized oocytes, and have sperm that fail to activate *in vivo*. Mutant males are fertile and produce cross progeny with mutant hermaphrodites. Mutant male sperm are unable to activate in the presence of known *in vitro* activator zinc sulfate and have variable morphology in *in vitro* activators pronase and proteinase K. Mutant male sperm are motile and migrate to the spermatheca *in vivo.* These *spe-57* phenotypes resemble those of the SPE-8 group, suggesting that *spe-57* is implicated in the SPE-8 pathway. SPE-6 is an intracellular kinase that is an inhibitory regulator downstream of both the SPE-8 and TRY-5 activators. Epistatic analysis showed that *spe-6(hc163)* suppressed the fertility defects of *spe-57(tm5344)*, therefore indicating that *spe-57* is upstream of *spe-6*. SPE-57 is sperm-specific and has been shown to localize strongly to the cell surface of spermatocytes, spermatids, and spermatozoa. Predicted AlphaFold structures suggest that SPE-57 is a Ser/Thr phosphatase and a single pass transmembrane protein. Future work will focus on the detailed cellular defects of sperm activation in *spe-57(tm5344)* mutants and explore the regulatory mechanism of SPE-57. Together, our work identifies *spe-57* as a new phosphatase involved in the regulation of sperm activation.

355B Multi-pathway post-transcriptional regulation of SCFPROM-1 in *C. elegans* germline.

Ariz Mohammad[1], Hieu Hoang[2], Vincent Gao[1], Britney Sun[1], Verena Jantsch[3], Tim Schedl[11]Genetics, Washington University in St Louis, [2]Pediatrics, Washington University in Saint Louis, [3]Chromosome Biology, University of Vienna

The entry of germline stem cells (GSCs) into meiotic prophase is a key event in germ cell development. Three redundant posttranscriptional pathways promote the switch, GLD-1, GLD-2 and SCFPROM-1. SCFPROM-1 is an E3 ubiquitin ligase complex that degrades substrate proteins at meiotic entry that are expressed in the progenitor zone (PZ), where PROM-1 is the F-box substrate specificity subunit. SCFPROM-1 controls meiotic entry by repressing mitotic cell cycling, at least in part by degradation of cyclin E, and activation of the CHK-2 kinase, a major regulator of meiotic entry that promotes homologous chromosome pairing, synapsis, and DSBs. Nuclear-localized PROM-1 is undetectable in the GSC region of the PZ, then increases in a sigmoidal pattern to peak at the start of meiotic prophase. To identify factors regulating PROM-1, we assayed mRNA and protein levels in the germline of mutants of known regulators of meiotic entry and early meiotic prophase. We found that GLD-2 pathway genes, GLD-2 and GLD-3, act as activators of PROM-1 accumulation for peak levels at meiotic entry. GLD-1 pathway genes GLD-1, and NOS-3, act as inhibitors of peak PROM-1 accumulation. Interestingly, loss of genes in both pathways result in reduced PROM-1 throughout the distal germline. Moreover, GSC regulator, FBF-2 (but not GLP-1\FBF-1) is an activator of PROM-1 accumulation. While *prom-1* mRNA is transcribed in both the PZ and meiotic prophase, cytoplasmic mRNA levels mimic protein levels i.e. very low in the GSC region and then increases to peak at meiotic entry. Cytoplasmic mRNA levels are also correlated with PROM-1 protein levels in the *gld-1* and the *gld-2* pathway mutant germlines, suggesting that PROM-1 accumulation is achieved by stabilization of cytoplasmic *prom-1* mRNA. We found that CCF-1, a component of the CCR4-NOT mRNA degradation complex, is responsible for the degradation of *prom-1* mRNA in GSCs. However, higher cytoplasmic *prom-1* mRNA in the distal-PZ of *ccf-1(lf)* germlines did not result in increased PROM-1, suggesting another level of regulation, translational repression, that further protects GSCs from precocious PROM-1 expression and premature differentiation. Swapping 3′ UTR of *prom-1* with *tbb-2* 3′ UTR results in lower protein levels (~50% of peak) throughout, although slightly increased PROM-1 in GSC region, suggesting non-3′UTR mediated control as yet another level of regulation of PROM-1. Findings from follow up investigations will be presented at the meeting.

356B Machine-learning-assisted analysis of *C. elegans* developmental parameters using a high-throughput microfluidic technology

Sudip Mondal[1], Andrew DuPlissis[1], Abhishri Medewar[1], Evan Hegarty[1], Adam Laing[1], Amber Shen[1], Sebastian Gomez[1], Adela Ben-Yakar[21]vivoVerse, [2]The University of Texas at Austin

Analyzing developmental parameters is essential for numerous biological studies involving whole organisms. In particular, assessing developmental toxicity from chemical exposures requires careful evaluation of organismal development. While traditional testing methods mainly rely on large mammalian models, *C. elegans* has emerged as a promising new approach methodology (NAM) due to its biological relevance and suitability for high-throughput studies. However, current low-resolution and labor-intensive techniques limit its application in sub-lethal developmental toxicity studies at high throughputs. The recent development of the large-scale microfluidic device, vivoChip, enables the rapid acquisition of 3D high-resolution images of approximately 1000 *C. elegans* from 24 distinct populations. To complement this fast data collection, we developed a machine learning (ML)-based image analysis platform called vivoBodySeg, which employs a 2.5D U-Net architecture to accurately segment *C. elegans* bodies. vivoBodySeg processes 36 GB of data per device, achieving a Dice score of 97.80% and phenotyping multiple body parameters within just 35 minutes on a standard desktop PC. This ML-driven approach provides highly reproducible body parameter measurements (4–8% coefficient of variation) and enables the robust assessment of chemical toxicity with high statistical power.

357B Spatial control of expression of the LIN-31/FOXB transcription factor and exploration of its role in VPC fate patterning

Snigdha Musugunthan[1], Qi Zhang[1], David Reiner[1,21]IBT/HSC, Texas A&M University, [2]College of Medicine, Texas A&M University

Vulval precursor cells (VPCs) comprise an equivalence group of six specialized epithelial cells, adopting fates in a 3˚-3˚-2˚-1˚-2˚-3˚ pattern in response to EGF signal from the Anchor Cell. The LIN-31/FOXB winged helix transcription factor acts as the terminal selector for VPCs. LIN-31 and LIN-1/ETS are repressed by the Ras>MAP Kinase pathway, and in turn they suppress vulval fates. We published a proof-of-concept transcriptomic analysis of VPC development using PAT-seq (Poly(A) Test RNA-sequencing). By expressing GFP::FLAG-tagged poly-adenylation binding protein (PAB-1), under control of the published lin-31 promoter, we immunoprecipitated FLAG from mixed stage animals and sequenced associated transcripts, identifying VPC-expressed genes. While GFP from these transgenes was expressed in VPCs, it also appeared in unidentified head and tail cells. A CRISPR/Cas9-mediated GFP insertion at the 5′ end of the endogenous lin-31 locus confirmed this expression pattern, highlighting the need to identify VPC-specific regulatory sequences. To refine VPC-specific lin-31 regulation, we are systematically deleting non-coding sequences. First, we are modifying the published lin-31 promoter in GFP reporter constructs and reinserting them into animals via recombinase-mediated cassette exchange (RMCE). Second, we are deleting upstream regulatory sequences in GFP::LIN-31 animals via CRISPR. Our initial deletion of 3.9 kb upstream of the DNase hypersensitivity region (DHS), characteristic of open chromatin, did not confer the Multivulva phenotype characteristic of lin-31 loss of function, suggesting that essential VPC-specific regulatory elements lie beyond this region or within the large introns of lin-31. We are also investigating lin-31 transcriptional regulation by perturbing key VPC competence factors (LIN-39/Hox, BAR-1/β-catenin, PRY-1/Axin) and signaling pathways (Ras>MAPK, Notch) to assess their impact on expression of GFP::LIN-31. Additionally, we will disrupt lin-31 via CRISPR to examine its effects using fluorescent markers for 1˚, 2˚, and 3˚ VPC fates. This study aims to refine our understanding of the role of LIN-31 in VPC development while also refining transcriptomic tools to explore impacts of signaling on the VPC genomic landscape.

358B Structure-Function Analysis of the LET-99 Protein During Asymmetric Division

Laurel Koch, Y Vy Nguyen, Alan Rose, Lesilee Rose Molecular and Cellular Biology, University of California, Davis

Asymmetric cell division generates cellular diversity by producing daughter cells with distinct identities and is important for normal development. During many asymmetric divisions, a cell polarity axis forms with an unequal distribution of cell fate determinants, and the division plane must be coordinated with this axis to ensure the correct inheritance of determinants. The division plane is determined by the precise positioning of the nucleus and mitotic spindle. Conserved PAR polarity proteins regulate asymmetric division in many organisms, by controlling the localization of downstream effectors. In the *C. elegans* embryo, the LET-99 protein is essential for orientation of the spindle onto the polarity axis in the one cell embryo and in the P1 cell at the two-cell stage. In these cells, anterior and posterior PAR proteins occupy distinct domains. The PAR proteins inhibit LET-99's localization at the membrane, restricting LET-99 to a lateral posterior band. LET-99 in turns inhibits the localization of the GPR-1/2 and LIN-5 components of the force generating complex, creating unequal microtubule pulling forces on astral microtubules that orient the spindle. However, the regions of LET-99 required for its localization and its role in spindle positioning are unknown. LET-99 is a member of the DEPDC1 family of proteins, which contain an N-term DEP domain and a region with weak homology to Rho-GAP proteins. The DEP domains of several proteins recruit them to the membrane. LET-99 also contains predicted phosphorylation sites for the anterior PAR kinase PKC-3 and the posterior PAR-1 kinase. To determine which LET-99 regions are required for membrane localization or asymmetry, LET-99::mKate transgenes with deletions or with mutations of all six predicted PKC-3 sites (LET-99(6S>A)::mKate) were created. These transgenic proteins are being analyzed for their localization and ability to rescue the *let-99* mutant defects. Examination of one-cell embryos revealed that the first 300 amino acids including the DEP domain is not essential for membrane localization or LET-99's band pattern, but may be required for spindle orientation. We are currently examining the localization pattern of other deletions and of LET-99(6S>A)::mKate to identify sequences necessary for LET-99 asymmetry and regulation of spindle orientation at both the one and two-cell stages.

359C Temporal Scaling Properties of Cell Cycle Phases During Embryogenesis

Neil Peinado, Pavak Shah Molecular, Cell and Developmental Biology, University of California, Los Angeles

C. elegans embryos develop over a wide range of viable temperatures with embryogenesis either slowing or accelerating at low and high temperatures respectively and total cell cycle times lengthening or shortening in response. These changes occur without introducing errors to cell fate or viability despite substantial changes to cell cycle lengths across the range. In an apparent paradox, silencing cell cycle regulators via RNAi induces deleterious fate transformations and changes to cell cycle timings that are within those bounds caused by temperature variation. What phenomena maintain the fidelity of fate across temperatures that is disrupted in genetic perturbations to the cell cycle? We asked whether alteration to the timing of individual cell cycle phases, rather than total cycle time, might explain how fate is maintained across temperatures while being lost in genetic perturbations. Thanks to the efforts of Zhongying Zhao's group, a modified fluorescence ubiquitin cell cycle indicator (FUCCI) system is available for tracking cell cycle phase dynamics in C. elegans embryos. In this study, I utilize FUCCI expressing embryos to assess how cell cycle phase dynamics are altered in response to temperature variation in comparison to those seen in genetic perturbations to cell cycle regulation. Preliminary analysis comparing FUCCI bearing embryos grown at 21°C and 14°C reveal that S and G2 phases in the E lineage scale uniformly with total cycle time. In contrast, embryos treated with RNAi targeting a key cell cycle regulator demonstrate altered phase proportions. Thus, the maintenance of proper fate patterning in the embryo may depend on the maintenance of cell cycle phase durations within a reasonable bound of variation.

360C Is the intracellular domain of LAG-2 ligand required for germline Notch signaling?

Yang Peng[1], Anushka B Patel[2], Caroline Goutte[1]1Biology, Amherst College, [2]Biochemistry & Biophysics, Amherst College

Notch signaling is a cell-to-cell communication mechanism that is critical for cell fate determination and differentiation. Ligand-receptor interaction triggers a conformational change in the Negative Regulatory Region (NRR) of the Notch receptor, thereby exposing it to proteolytic events that release the Notch intracellular domain from its membrane tether. The canonical model of Notch signaling includes endocytosis of ligand by the signaling cell as an essential step in generating the force necessary for NRR unfolding. Recently, however, Langridge et al. (2022) demonstrated that the *C. elegans* Notch receptors, LIN-12 and GLP-1, can be activated without Epsin-mediated ligand endocytosis. Their analysis suggests that the mechanical force required to activate the NRR domain of *C. elegans* Notch proteins is less than that of their Drosophila or mammalian counterparts. Here we turn to a different context than that analyzed by Langridge et al., to ask if endocytosis-independent activation is involved in the Notch-dependent proliferative fate of the *C. elegans* germline stem cells. In our analysis, fertility and germline progenitor zone measurements provide a read-out for successful Notch signaling between the signaling somatic niche cells and the responding germline progenitor stem cells. Using modified *lag-2* transgenes provided by the Greenwald lab (Langridge et al.2022), we find that GLP-1 can be activated successfully in larval germ cell progenitors even if the only LAG-2 protein available lacks an intracellular domain. These observations suggest that GLP-1 activation in germ cell progenitor cells can be achieved without ligand endocytosis. Interestingly however, the progenitor zone of larvae that express LAG-2 without an intracellular domain is smaller than that of larvae expressing intact LAG-2. This result suggests that the intracellular domain of the ligand may allow for a higher level of Notch activation. A possible requirement for endocytosis as a means of achieving maximum GLP-1 activation is consistent with Langridge et al.'s proposal that the GLP-1 protein may be tuned to a higher force threshold than LIN-12. We will compare this effect with that of a simple reduction in *lag-2* dosage. Overall, our results define an observable germline phenotype associated with the absence of the LAG-2 intracellular domain. We are currently extending the analysis of this defect by measuring the biological impact on gametogenesis and fecundity.

361C WEE-1.3 is required for proper chromosome segregation during *C. elegans* spermatogenesis

Shannon Pfeiffer, Aimee Jaramillo-Lambert University of Delaware

In the United States, unexplained infertility accounts for approximately 15% of infertility cases. Defects in eggs or sperm production can result in infertility. Meiosis, the specialized type of cell division that produces eggs and sperm, is a complex process that is tightly regulated. This regulation ensures that these gametes are functional. Though similar, female and male meiosis are distinct processes that are differentially regulated. The kinase WEE-1.3, which is conserved throughout eukaryotes, is a key regulator of both the mitotic and meiotic cell cycles. WEE-1.3 prevents cells from dividing prematurely by inhibiting the G2-to-M transition. In *Caenorhabditis elegans* spermatogenesis, several gain-of-function (gof) mutations in *wee-1.3* cause primary spermatocyte arrest, while oogenesis and mitosis are unaffected, indicating a distinct role for WEE-1.3 in spermatogenesis regulation. To determine the role of WEE-1.3 during male meiosis we are using auxin-inducible degradation to selectively deplete WEE-1.3 from the *C. elegans* male germline. High-resolution imaging of both fixed and live samples reveals that WEE-1.3 depletion induces severe chromosome segregation defects during spermatogenesis in both the male and hermaphrodite germline. Defects include DNA fragmentation and chromatin bridge formation, during meiosis I and II. These defects may be due to errors in spindle assembly, as WEE-1.3 depletion also disrupts centrosome numbers and the organization of spindle microtubules at metaphase I. Examining WEE-1.3 localization in the male germline revealed a perinuclear localization pattern throughout meiotic prophase. Once spermatocytes reach metaphase I, WEE-1.3 localization shifts to the centrosomes, suggesting a role for WEE-1.3 in organizing the spindle during male meiosis. Future work will test the hypothesis that WEE-1.3 regulates centriole duplication, determine how WEE-1.3 regulates other components of M-phase entry, and identify spermatogenesis-specific WEE-1.3 interactors.

362C Roles of PAR-1 and NTL-9 in apico-basolateral polarity in the intestine

Melissa Pickett[1], Colton Duke[2], Deniz Akpinaroglu[3], Jessica Feldman[41]Biology, The College of Idaho, [2]San Jose State University, [3]UC Berkeley, [4]Stanford University

Apico-basolateral polarity is essential for the function of the epithelial cells that form organ linings. Disruption of apico-basolateral polarity is associated with many diseases, including microvillus inclusion disease, polycystic kidney disease, and the progression and metastasis of cancers. The mechanisms underlying apico-basolateral polarity are likely to be redundant due to the importance of polarity for organ function. Indeed, loss of individual polarity proteins often has minor effects on the structure and function of epithelia. We are using the *C. elegans* intestine as a simple *in vivo* model to test the hypothesis that parallel redundant pathways involving PAR-1, LGL-1, UBA-2, and/or NTL-9 are required to establish and maintain apico-basolateral polarity. In previous work we found that intestine specific depletion of PAR-1 or LGL-1 alone did not alter polarity establishment or worm growth. However, in a preliminary RNAi screen, we found that intestine specific depletion of PAR-1 (PAR-1gut(-)) along with RNAi depletion of *lgl-1* slowed worm growth while PAR-1gut(-) worms treated with either *uba-2* or *ntl-9 RNAi* had arrested embryonic and larval development. We used CRISPR to endogenously tag NTL-9 with GFP and the ZF degradation domain. We found that NTL-9 localized broadly in the cytoplasm of embryonic cells and the adult gonad. Surprisingly, intestine specific depletion of NTL-9 resulted in developmental delay, suggesting the protein may have a role in intestinal development on its own. We are currently investigating the role NTL-9 has alone and are building strains to simultaneously deplete both NTL-9 and PAR-1 to understand if these proteins function in parallel in polarity establishment and/or maintenance.

363C Insulin signaling in the somatic gonad regulates dauer entry and contributes to coupling gonad development with the non-gonadal soma in *C. elegans*

Grace E Przybyl, Julia Wittes, Iva Greenwald Biological Sciences, Columbia University

The gonad develops from two somatic precursor cells, Z1 and Z4, of the gonad primordium present at hatching. Little is known about how gonad developmental progression is coordinated with the rest of the developing worm. We began exploring potential roles for IIS in this coordination because IIS is required to initiate post-embryonic lineages and mediates various nutritional checkpoints for developmental progression. IIS acts in the hypodermis to regulate reactivation of non-gonadal precursor cells after starvation, implying a distinct cellular focus for IIS regulation of the initiation of the Z1 and Z4 division and possibly the timing of later events.

We used the auxin-inducible degron (AID) and “Flexon” systems with tissue-specific Cre drivers to produce tissue-specific depletions of DAF-2 in the somatic gonad throughout the first larval stage. We did not observe an effect on gonadogenesis through the formation of the somatic gonad primordium; instead, surprisingly, we found that depletion in the somatic gonad yielded a highly penetrant dauer constitutive phenotype that is regulated via the canonical IIS pathway and its components, comprising DAF-2, AKT-1/2, and DAF-16/FOXO. Preliminary results indicated that laser ablation of the germline precursors suppresses dauer entry when DAF-2 is depleted from the somatic gonad. These results identify the somatic gonad as a unique cellular focus for IIS regulating dauer entry while the germline acts an intermediary to relay the state of the somatic gonad to the nongonadal soma. We propose two signaling models by which the somatic gonad acts as an endocrine organ to regulate the decision between continuous development and dauer diapause. A “satisfaction” signal from the somatic gonad that is suppressed by DAF-16 in unfavorable conditions, or a “distress” signal from the somatic gonad that is activated by DAF-16 in unfavorable conditions.

Using DAF-2::AID to deplete activity at later times in development, we also found that loss of insulin activity in the somatic gonad in the L2 can uncouple the development of the gonad from the non-gonadal soma and that loss of IIS activity in the hypodermis can cause overextension of the gonad arms.

This poster will be a companion to the presentation by Wittes, Przybyl and Greenwald.

364C Nutritional status-inked dual cleavage control of MYRF as a gating mechanism for *C. elegans* larval stage transition

Zhimin Xu, Zhao Wang, Xiaoting Feng, Yingchuan B QiShanghai Tech University

The temporal regulation of post-embryonic development remains an open question in developmental biology. In Caenorhabditis elegans, specific microRNAs are activated in the mid-to-late phase of each stage to downregulate present-stage-determining factors, thereby promoting temporal identity for the next stage. However, the mechanisms that trigger their activation remain unknown. Moreover, while microRNAs establish stage identity, they do not drive stage progression, suggesting that a fundamental principle governing stage transitions remains undefined.

Here, we identify Myelin Regulatory Factor (MYRF) as a key regulator of larval stage transitions. MYRF is a conserved transmembrane transcription factor that first localizes to the cell membrane and undergoes self-cleavage to release its N-terminal fragment (N-MYRF), which subsequently translocates to the nucleus to regulate gene expression. We show that MYRF cleavage occurs in an oscillatory manner, peaking mid-to-late in each larval stage. This process is tightly controlled by dual inhibitory mechanisms: intrinsic inhibition by MYRF's juxtamembrane (JM) region and extrinsic inhibition by the cytoplasmic tail (CCT) of the transmembrane protein PAN-1. Disrupting these inhibitory interactions leads to premature developmental transitions and severe defects, including lethality.

Furthermore, MYRF cleavage occurs uniformly across all tissues and appears to be influenced by the nutritional state of the animal, suggesting the presence of systemic signals that trigger cleavage. However, the exact nature of these signals remains unknown. Using inducible degradation of MYRF, we demonstrate its necessity for proper stage transitions and its role in modulating key components of the developmental timing network. These findings establish MYRF as a crucial gating factor that coordinates stage progression, uncovering a previously uncharacterized regulatory layer in developmental timing.

365C The kinesin KLP-20 directly interacts with miRISC component VIG-1 and regulates the *let-7* miRNA during epidermal morphogenesis

Dan C Quesnelle, Jeffrey Boudreau, Ian D Chin-Sang Biology, Queen's University

Morphogenesis, an organism's ability to generate proper body shape, is a crucial step in its development. Despite the importance of establishing correct body morphology, many of the molecular mechanisms that control this process remain elusive. Previous genetics have identified the kinesin motor KLP-20/KIF3A as a regulator of epidermal morphogenesis. KLP-20 is a subunit of the kinesin-II motor complex and is canonically involved in ciliogenesis. Loss-of-function mutations in the *klp-20* gene produce a variable bumpy epidermal phenotype that forms during late embryogenesis. Interestingly, *klp-20* is only expressed in the nervous system, meaning that it must be functioning cell non-autonomously to regulate epidermal morphogenesis.

A yeast two-hybrid screen identified miRISC component VIG-1 as a direct interactor with KLP-20, suggesting a role for KLP-20 in microRNA regulation. We show that *klp-20* regulates the miRNA *let-7* and its target *hbl-1*. In a *klp-20* mutant background loss of *let-7* enhances the bumpy epidermal phenotype and HBL-1 is expressed abnormally, suggesting that *let-7* is unable to function properly in animals with mutant *klp-20*.

The variability of *klp-20* mutant phenotypes pose a challenge, but we have created a fully-penetrant genetic background in which we can perform genetic screens to identify suppressors of the bumpy epidermal phenotype. Furthermore, this work may be the first to identify a role for *let-7* in the embryo as previous data indicates *let-7* is not expressed until the mid-larval stages. MicroRNAs are rapidly being identified as post-transcriptional regulators in a wide array of processes and thus understanding their role in morphogenesis is critical for our understanding of how gene expression is regulated during development as a whole.

366C Characterizing the role of *spe-57* in spermiogenesis through a temperature-sensitive allele *as47*

Allison Ramz, Emily Mincher, Benjamin Khaim, Noah Dickinson, Sara Nicaj, Zain Uddin, Xue Mei Saint John's University

Fertilization is a crucial process for all sexually reproducing organisms. To prepare for fertilization, the gametes need to develop and differentiate properly. The nematode *Caenrohabdtis elegans (C.elegans)* serves as a model organism for studying fertilization and gametogenesis. In *C.elegans*, spermatids undergo post-meiotic differentiation and transition into motile and mature spermatozoa capable of fertilizing oocytes. This project aims to examine sperm activation in a temperature sensitive mutant *as47*. This allele was isolated through forward mutagenesis screening and showed subfertility at 25˚C. The gene affected in *as47*, *R03D7.8*, now named *spe-57*, encodes a Ser/Thr phosphatase. Here we use *in vitro* sperm activation to characterize the mutant. *In vitro* sperm activation is a process by which external reagents activate spermatids outside of the worm's bodily mechanisms. *In vitro* activators such as Pronase, Proteinase K, and zinc sulfate all induce the formation of pseudopods, similar to sperm activation in vivo. Differential interference contrast (DIC) microscopy provided visualization of spermatids undergoing activation and individual pseudopod growth. As standard conditions, wild-type sperm undergo *in vitro* activation and are shown to be 80% activated in Pronase, 85% activated in Proteinase K, and 80% activated in zinc sulfate. The mutant *as47* showed 30% spike formation in Pronase and an occasional pseudopod. *as47* showed 60% activation in Proteinase K and was not seen to be activated in zinc sulfate. These defects mimic those of the *spe-8* group mutants, suggesting the gene *spe-57* acts in the *spe-8* pathway that regulates sperm activation. Further experimentations will focus on the mechanism by which the *spe-57* gene regulates sperm activation.

367C Exploring Connections between Dystrophinopathies and Autism Spectrum Disorder: Investigating the Role for *dys-1* in *C. elegans* Social Feeding Behavior

Ava G Remoll[1], Ryan Trenholm[2], Brett Warden[2], Gili Butler-Furlong[1], Casey Ellison[1], Olivia Davir[1], Bailey June Hamm[1], Te-Wen Lo[1], Abbi Paulson[21]Biology, Ithaca College, [2]Mathematics and Natural Sciences, Elmira College

Autism is a neurodevelopment disorder that affects social interaction, communication, and behavior. Of interest, about 19% of patients with dystrophinopathies, caused by mutations in the dystrophin gene, are reported to have autism1. Dystrophin links the muscle cell actin cytoskeleton to the extracellular environment, facilitating signal reception necessary for motion2. Dystrophin also has roles in Purkinje cells of the cerebellum (coordination and muscle tone), and Schwann cells (myelination)1. We are utilizing *C. elegans* to better understand the role of dystrophin in autism spectrum disorder (ASD). *C. elegans* has one ortholog of the human dystrophin gene, *dys-1*, expressed in body wall muscle, head, pharynx, and vulva3,4. Mutations in some *C. elegans* autism-associated ortholog genes have been reported to alter social feeding behaviors5. To examine the link between muscular dystrophy and ASD, we have been studying if dystrophin knockdown also affects the social feeding behavior of *C. elegans*.

Feeding RNA interference was used to deplete *dys-1* expression, and social feeding assays6 performed. Experiments were conducted in two strains: N2 and CB4932, which has increased social feeding behaviors compared to N2. Worms were assessed for bordering (worms within 2 mm of the lawn edge) and clumping (worms touching others along 50% or more of their body length). In CB4932 mutants, *dys-1(RNAi)* did not affect bordering in comparison to *L4440(RNAi)* controls (averages: 61% and 64%, n=4), however there was a 14.4% decrease in clumping (averages: 38.2% and 23.8%, n = 4). No significant differences were observed between N2, *dys-1(RNAi)* treated (10.5%, n=3) and *L4440(RNAi)* treated (9.53%, n=3) *C. elegans.* This indicates that *C. elegans* could be a productive model for examining the link between autism spectrum disorder and dystrophinopathies. Future directions could include determining where and when *dys-1* expression is needed to promote social feeding and the molecular mechanisms involved.

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368C Histone methyltransferases differentially regulate transcription and chromosome structure in oogenesis and spermatogenesis

Carolyn Remsburg[1], Lacole Fung[1], Lauren Salvitti[2], Aimee Jaramillo-Lambert[11]Biological Sciences, University of Delaware, [2]Charles Rivers Laboratory

Meiosis is the specialized cell division that results in the formation of haploid gametes from a diploid cell. During meiosis, duplicated chromosomes must undergo significant condensation and compaction, which are regulated by condensins, cohesins, and post-translational histone modifications, before segregating into daughter cells. Defects in this process can lead to aneuploidy and are a leading cause of miscarriages and birth defects. Our lab identified that MET-2, a histone methyltransferase that catalyzes the dimethylation of histone H3 lysine 9 (H3K9me2), differentially impacts chromosome length in the male vs. female *C. elegans* germline. In *met-2* null worms, chromosome length during spermatogenesis is significantly shorter than wild type (WT), while chromosome length during oogenesis is not significantly different than WT. Interestingly, preliminary data suggests that the single X-chromosome in males, which is typically highly enriched in H3K9me2 at diakinesis, is not shorter in *met-2* males compared to WT males, suggesting that MET-2 only regulates the length of autosomes during spermatogenesis. In male worms harboring a mutation in the catalytic domain of MET-2 (*met-2* CD), chromosome length is not changed compared to WT suggesting MET-2 plays a noncatalytic role in mediating chromosome structure. However, *met-2* and *met-2* CD worms have reduced brood sizes, indicating reduced gamete quality. Immunolabeling shows that MET-2 localizes to germline nuclei from the distal tip through diplotene in hermaphrodites. In males, MET-2 localization persists through diakinesis suggesting that MET-2 may function later during spermatogenesis. As H3K9 methylation is associated with transcriptional repression, we assayed for global transcription by immunolabeling for active RNA pol II. *met-2* males have more active RNA pol II in later stages of meiosis, indicating that changes in transcription may contribute to the reduction of gamete quality. RNASeq performed on *met-2* male gonads indicate that genes on the X chromosome are significantly more likely to be upregulated than genes on autosomes, even though X-univalent length is unchanged. In addition, several genes that regulate meiosis and gamete quality, such as *gon-14, oma-2* and *msp-152* are upregulated in *met-2* male gonads. These results suggest that MET-2's role is not limited to its enzymatic activity, but rather MET-2 has multiple functions during meiosis.

369C Intestinal morphology guides the placement of the gonad along the left-right body axis

Bryn Romig, Ian Larkin, Greg Hermann Lewis & Clark College

The *C. elegans* hermaphrodite gonad displays prominent asymmetries along both the anterior-posterior and left-right body axes. While much is known regarding the developmental process generating the proximal-distal asymmetries within the gonad that align with the anterior-posterior body axis, we have a more limited understanding of how the gonad becomes positioned asymmetrically along the left-right body axis. The gonad primordium is composed of 4 cells, Z1-Z4, that are initially aligned symmetrically along the left-right body axis during mid embryogenesis. At hatching, the L1 stage gonad primordium is asymmetrically positioned is so that Z1/Z2 are located anteriorly along the right side and Z3/Z4 are located posteriorly along the left side of the body. In the adult hermaphrodite, the anterior gonad arm, resulting from the proliferation of Z1 and Z2, lies along the right side of the body and the posterior gonad arm, which is derived from Z3 and Z4, lies along the left side of the body. The mechanisms guiding the asymmetric positioning and morphogenesis of the gonad relative to the left-right axis are unknown. Given the close association between the gonad primordium and the intestine during both embryonic and post-embryonic development, it is likely that interactions between these two organs contributes to asymmetric gonad positioning along the left-right axis. We will present work characterizing the effects of a mutation altering intestinal cell behavior and morphology that impacts gonad development and causes mispositioning of the gonad along the left-right body axis.

370C Searching for Asymmetry During the Asymmetric Division of the EMS Cell

Lesilee Rose, McKenzi Fernholz, Jennifer Baily, Alan Rose Univ California, Davis

Asymmetric cell division produces daughters with different fates and is essential for development. During many asymmetric divisions, the mitotic spindle is oriented along a polarized axis, so that division differentially partitions cell fate determinates to the daughter cells. In the one-cell *C. elegans* embryo and many systems, the spindle is oriented onto the PAR protein polarity axis via a conserved force generating complex, consisting of LIN-5/NuMA and dynein. In contrast, the spindle does not align with the PAR axis in the EMS cell of the four-cell *C. elegans* embryo; rather, partially redundant Wnt and MES-1/SRC-1 signaling from EMS's neighbor, P2, appear to converge on LIN-5 and dynein to orient the spindle on the anterior-posterior axis and move it towards the EMS/P2 cell contact. Our lab previously identified additional factors involved in the MES-1/SRC-1 pathway including LET-99, a protein known to regulate the force generating complex in the one-cell, the Rac protein CED-10, and the branched actin regulator ARX-2. Genetic studies are consistent with the model that LET-99 acts downstream of MES-1 and other factors. LET-99 cortical levels appear to be reduced at the EMS/P2 cell contact, while ARX-2 and LIN-5 are enriched at the EMS/P2 cell contact compared to other cell contacts. We examined GFP::LIN-5 in *let-99* and *mes-1* mutants and found that LIN-5 was less enriched at the EMS/P2 contact in both cases. However, MES-1 is also involved in spindle orientation in the P2 cell, and the limits of light microscopy preclude determining if a cortical protein is present in the EMS cell or its neighbor. To circumvent this issue, we generated embryos expressing photo-convertible Dendra2::LET-99, for single-cell specific conversion and visualization of the protein. We found that Dendra2::LET-99 localization is indeed asymmetric: LET-99 is present at high levels on the EMS cortex except where EMS contacts P2. Preliminary analysis of Dendra2::LIN-5 photoconversion in EMS or P2 indicates that LIN-5 has the highest levels at the EMS/P2 cell contact in both cells. We are currently testing whether LIN-5 asymmetry is dependent on either MES-1/SRC-1 or Wnt signaling.

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372C Investigation of ABCF-1 as a Regulator of *C. elegans* Developmental Protein Aggregation

Daniela Santos Da Mata[1], Bryan Phillips[21]Biology, University of Iowa, [2]University of Iowa

Biomolecular condensates play a crucial role in post-transcriptional and post-translational regulation. These granules form through protein-protein and protein-RNA interactions, and can be organized into dynamic liquid droplets, less soluble hydrogels, or more solid amyloid oligomers and fibers. Condensates are found in both somatic and germ cells, varying in function and composition. For instance, a specific type of condensate known as P-body is present in both somatic and germ cells under stress conditions such as heat shock or osmotic stress, as well as under physiological conditions. Additionally, P-bodies mediate translational repression and mRNA decapping. Given their important role, the regulation of condensate assembly and disassembly is essential. One proposed regulatory mechanism involves chaperone-based disaggregases, such as the Hsp104 in yeast or the Hsp110/70/40 complex in metazoans. Evidence also suggests that the ABCF proteins, members of the ATP-binding cassette (ABC) superfamily, have a disaggregase activity in yeast and worms and thus represent an understudied candidate for condensate regulation. Specifically, loss of function of the chaperone ABCF-1 in *Caenorhabditis elegans* resulted in amyloid aggregation and germline defects. However, whether ABCF-1 is indeed a bona fide disaggregase and the mechanisms by which it regulates germline function remain unknown. In this study, we demonstrate that ABCF-1 regulates P-bodies by preventing the excessive accumulation of P-body components and maintaining their solubility. We found that RNAi-mediated knockdown of ABCF-1 induces sterility in *C. elegans* and leads to the formation of puncta containing P-body proteins such as CAR-1, MEX-3, and PAB-1 in the germline. Moreover, co-staining for CAR-1 and MEX-3 during ABCF-1 loss of function revealed that these proteins colocalize. Our results demonstrate that ABCF-1 maintains P body solubility, and its loss of function results in the aggregation of P body components. CAR-1 and MEX-3 colocalization suggests that these aggregates may still function as P-bodies rather than forming toxic accumulations, implying that the germline dysfunction may result from hyperactive P-bodies. Overall, our findings provide new insights into mRNA regulation during development and highlight the role of condensates in early *C. elegans* development.

373C Decoding vulval cell identity: Insights from single-nucleus RNA Sequencing

Helen F Schmidt, Christopher L Large, John I Murray, Meera V Sundaram Genetics, University of Pennsylvania

The *C. elegans* vulva is an epithelial tube composed of 22 cells classified into 7 cell types, each with distinct shape and position. The vulva is a well-studied model for signaling and cell type specification - EGF-Ras-ERK specifies primary (1˚) lineage-derived cells (vulE/F) while Notch specifies secondary (2˚) lineage-derived cells (vulA/B1/B2/C/D). The 7 vulva cell types differ in the expression of some known marker genes. However, the full range of differences and the role of each cell type in vulva function are less clear. To better understand the biological distinctions between vulval cell types, we performed single-nucleus RNA sequencing (snRNA-seq) of vulval and other epithelial cells throughout the L4 larval stage when the vulva tube develops. Our analysis revealed that different epithelial tissues and specific vulval cell types differ significantly in their expression of apical extracellular matrix (aECM) components, such as collagens and Zona Pellucida domain proteins. This suggests producing a distinct aECM is a key aspect of epithelial cell identity. We also identified novel patterns of cell type-specific transcription factor expression, providing possible links between known targets of signaling pathways and the transcriptome of each cell type. Finally, we compared the transcriptomes of vulva cells from *egl-38*/Pax transcription factor mutant and wild type worms. We identified differences specifically in the vulE/F cells, which express EGL-38. Overall these datasets reveal clear differences among vulva cell types and will support continued exploration of the vulva as a model for epithelial cell identity and function.

374C A sequencing-based, whole animal screening method identifies regulators of EGFR signaling in *Caenorhabditis elegans*

Hillel Tsvi Schwartz, Paul Sternberg Biology and Biological Engineering, California Institute of Technology

Suppressor screens can identify genetic modifiers of biochemical pathways but highly efficient screens generally require that the suppressed mutant be viable and fertile. We developed an aclonal mutagenesis screening method that obviated this requirement and enabled the identification of mutations that partially suppressed the early larval lethal phenotype caused by loss of the *Caenorhabditis elegans* epidermal growth factor (EGF) receptor ortholog LET-23. We chemically mutagenized animals carrying the likely null allele *let-23(sy15)* and recovered *let23(sy15)* homozygotes that had escaped early developmental arrest and paralysis but were not viable. These animals were converted into genome sequence without first establishing mutant lines. Sequences of 68 animals with a delayed lethal phenotype identified 4,154 coding changes. Subsequent testing of individual candidate causal mutations using genetic engineering and the analysis of existing mutants identified 11 genes that mutated to mitigate early lethality caused by loss of EGF signaling. Among these were genes encoding homologs of the small GTPase Ras (*let60*), the tumor suppressor MSH4 (*him-14*), and several regulators of the small GTPase Rho, including homologs of the RhoGEF PLEKHG6 (*rhgf-2*), the scaffolding protein PATJ (*mpz-1*), and the phosphotyrosine interactor TENSIN (*tns-1*). Epistasis experiments and tissuespecific rescue were consistent with the repression of Rho activity or the activation of DNA damage response components compensating for the loss of EGF signaling and RAS pathway activity. Aclonal screening is adaptable to a range of organisms and would enable the identification of any mutation for which the phenotype does not allow the recovery of viable animals.

375C Characterizing the Transdifferentiation of the Steroidogenic Neuroendocrine Cell XXX in *C. elegans*

Njeri Z. R. Sparman[1], Pushkar Shinde[2], Oliver Hobert[1,3]1Department of Biological Sciences, Columbia University, [2]Columbia University, [3]Howard Hughes Medical Institute

In unidirectional cellular differentiation, a progenitor cell of broad potential gives rise to successively more determined daughters which, at each division, are more epigenetically restricted in their potential terminal state. Once a final functional state has been achieved, a postmitotic cell conventionally maintains that fate. Direct transdifferentiation is the process by which a normally quiescent terminally differentiated cell assumes a different terminal state without dividing. In the model nematode *C. elegans*, several neurons in both sexes and some male-specific neurons arise from the transdifferentiation of glial or epithelial cells. The neuroendocrine XXX cells are a pair of steroidogenic cells responsible for maintaining reproductive development of the larval worm by secreting the steroid hormone dafachronic acid to block entry into an anatomically distinct "dauer" larva under favorable conditions. The two XXX cells begin as functional constituents of the embryonic hypodermis, after which both cells delaminate from the surface and migrate ventrally and posteriorly to a final position between the bulbs of the pharynx in the head of the L1 larva, where they adopt an unusual star-shaped morphology. We aim to characterize this process in greater detail and unravel the mechanisms of this transdifferentiation event. First, a suite of fluorescent promoter fusions, and transcriptional and translational reporters are being developed to visualize gene expression and cellular morphology defining hypodermal, terminal, and intermediate states. Second, based on known expression profiles of homeodomain transcription factors, we use a candidate gene approach to define putative terminal selectors of XXX. We found that the *ceh-8* homeobox gene is indeed required for the expression of several molecular markers of XXX identity. Third, other factors unique to the transdifferentiation of XXX are being identified via unbiased EMS mutagenesis screening to saturation for loss of reporters of XXX terminal fate.

376C Investigating the association between FBF-1 function and germ granule compartment organization in *C. elegans* germlines

Camille K Spencer, Gabriella Weiss, Ekaterina Voronina University of Montana

Stem cells are essential for tissue maintenance through differentiation and proliferation to generate new cells. FBF proteins are key regulatory proteins that control germline stem cell dynamics in C. elegans. In C. elegans germline stem cells, FBFs are found in germ granules, specialized organelles within the germline cytoplasm that preserve germline identity. The localization of these granules is generally conserved among species, but the mechanisms regulating their specific substructure are not completely understood.

Recent developments suggest that FBF-1 facilitates epigenetic small-RNA inheritance associated with germ granules, and functional differences can be observed in mutated strains where FBF-1 is present. We hypothesize that the changes in function observed in FBF-1 mutants are directly associated with substructural changes within germ granules.

Using a standard cross strategy, we generated two mutated strains of C. elegans, combining the markers of germ granule compartments with fbf-1(ok91) loss of function. Using immunostaining, we observed that both WAGO-4-marked Z granules and MUT-16-marked Mutator foci formed in fbf-1(lf), but their relative distances from the P granule compartment of the germ granule differ from the WT, and MUT-16 formed abnormal puncta.

This suggests that FBF-1 may directly or indirectly control the assembly of some of germ granule subcompartments or impact orientation of granules relative to one another. Further, FBF homologs may control small-RNA regulation in other species in which this mechanism is conserved.

377C Collagen-Mediated Regulation of Distal Tip Cell Migration Cessation in *Caenorhabditis elegans*

Victor Stolzenbach[1], Sophie Griffin[2], Priti Agarwal[3], Ronen Zaidel-Bar[3], Erin Cram[11]Biology, Northeastern University, [2]Northeastern University, [3]Cell and Developmental Biology, Tel Aviv University

The distal tip cell (DTC) of *Caenorhabditis elegans* guides gonad development, transitioning from a migratory state in larval stages to a stationary state in adulthood. To identify the transcriptional changes that underlie this shift, we analyzed an RNA-seq dataset from isolated DTCs to characterize the patterns of gene expression that underlie the cessation of DTC migration and prevent continued gonad elongation. Utilizing a variety of bioinformatic tools, we identified genes involved in transcriptional regulation, signaling, extracellular matrix, and transmembrane transport. Among extracellular matrix components, collagens emerged as a major group of differentially expressed genes, with many showing significant changes between larval and adult DTCs. Given their structural role and involvement in tissue remodeling, we hypothesized that specific collagens contribute to stabilizing the adult DTC and maintaining gonad architecture. To test this, we performed a targeted RNAi screen and identified several collagens required for proper DTC migration cessation. In addition to migration defects, knockdown of some collagens resulted in altered gonad morphology, including changes in gonad width, suggesting broader roles in tissue organization. These results suggest specific collagens play a role in the cessation of gonad elongation and in maintaining gonad integrity. Because collagen remodeling is a hallmark of cancer metastasis and fibrotic diseases, this study has implications for the regulation of cell migration in development and disease.

378C Identifying new cell fate conversion barriers in *C. elegans*

Marcel Studt, Taina Averdieck, Hanna Heydeck, Caroline Klauer, Lara Kurzok, Baris Tursun University of Hamburg

Direct reprogramming by overexpressing transcription factors (TFs) is a promising strategy for producing cells for therapeutic purposes.

Understanding cell fate regulation mechanisms in cellular reprogramming is essential for developing strategies to improve cellular reprogramming and ensure the safety of reprogrammed cells.

In collaboration with the Pereira Lab at Lund University, we screened for new candidate genes that act as cell fate safeguards. The Pereira lab identified regulators of plasticity and cellular potency in human stem cells. We are testing corresponding orthologs in *C. elegans* for similar roles during direct reprogramming. To test their effects on cell fate reprogramming, we knock down genes via RNAi while simultaneously overexpressing the zinc-finger transcription factor CHE-1, which specifies ASE neurons during development. Transgenic animals that express the ASE neuron fate reporter *gcy-5*::*GFP* allow us to examine for ectopic ASE fate induction.

Interestingly, the Pereira lab list includes several cell fate safeguards that we already identified as reprogramming barrier genes in a previous screen from our lab (Kolundzic et al., 2018) using CHE-1 TF-induced reprogramming to neurons. Yet, a few additional candidate barrier genes that were not included in our list could be identified.

Overall, the remarkable conservation of reprogramming barriers highlights the power of *C. elegans* as a gene discovery tool in the context of biomedical research.

379C CWN-2/Wnt localization on seam cells challenges gradient-dependent polarity regulation by Wnt

Samal Tazhibayeva[1,2], Hitoshi Sawa[1,21]National Institute of Genetics, [2]Department of Genetics, The Graduate University for Advanced Studies, SOKENDAI

Cells rely on directional cues to navigate in space, polarize appropriately, establish body axes, and form functional structures. This navigation system is governed by morphogens, which are widely believed to form diffusion gradients, which is then translated by cells into positional information.

In *C. elegans* Wnt regulates the orientation of polarized cells. However, how cells compare concentration differences of extracellular molecules diffusing over long distance is poorly understood. Moreover, while Wnt distribution was shown to be graded in *C. elegans*, direct evidence for gradient formation of freely diffusing Wnts is lacking. Therefore, the precise nature of the information conveyed by Wnt to direct cell polarity remains unclear.

To address this question, we use seam cells as a model. Seam cell polarity is redundantly regulated by three Wnts: EGL-20 and CWN-1 secreted from the posterior region, and CWN-2, secreted from the anterior region; and their receptors — MOM-5/Fzd, LIN-17/Fzd and CAM-1/Ror (Yamamoto et al. 2011). Our lab previously reported that posteriorly secreted Wnts and their receptors asymmetrically localize to the posterior side of the seam cell membrane, and that this localization is reciprocally regulated, suggesting a positive feedback-based mechanism (IWM 2019).

Surprisingly, however, we found that anteriorly secreted CWN-2 also localizes to the posterior seam cell membrane, contradicting its expected gradient. This localization is unaffected in posterior Wnt mutants, indicating that CWN-2 posterior localization is independent of posterior Wnts. This finding suggests the existence of a gradient-independent mechanism for polarity orientation.

Additionally, we discovered a unique role of CAM-1/Ror regulating CWN-2 localization through its kinase domain. Our lab previously showed that CAM-1 exhibits an anterior-to-posterior expression gradient. Since CWN-2 localization depends on CAM-1, the observed CWN-2 localization gradient may not reflect its actual diffusion gradient, but rather mirror the CAM-1 expression gradient. This raises further questions about whether Wnts truly form gradients as they diffuse. To test this, we are using an inducible GFP-nanobody-based morphotrap system to visualize the distribution of freely diffusing Wnt molecules, challenging the conventional gradient model.

380C TDRD-3: A Novel Tudor Domain Protein Linking P Granules and Stress Granules in *C. elegans*

Sushmita Thakuri, Patricia Giselle Cipriani, Kris Gunsalus, Fabio Piano Biology, New York University

Ribonucleoprotein (RNP) granules are membraneless organelles composed of RNA and RNA-binding proteins with disordered regions that promote phase separation into condensates (Banani et al., 2017). Germ granules (GGs) and stress granules (SGs) are two conserved mRNP granules that, despite distinct functions, share similarities in composition and assembly (Brangwynne et al., 2009; Parker et al., 2016; Hofmann et al., 2021; Phillips and Updike., 2022). Both contain DEAD-box RNA helicases, translation initiation factors, and Argonautes proteins, and they are associated with mRNA sequestration and low translation output (Parker et al., 2016; Phillips and Updike., 2022; Pamula et al., 2024). However, how they coordinate mRNA regulation remains unclear.

Using *C. elegans*, we identified Y50D4C.3 as a novel GG and SG component. BLAST searches indicate that Y50D4C.3 is orthologous to human, murine, and Drosophila TDRD3; hence, we have named Y50D4C.3 as *tdrd-3*. TDRD3 is a Tudor domain protein involved in transcriptional co-activation and SG assembly (Yang et al., 2010; Goulet et al., 2008). While ubiquitously expressed throughout the mammalian tissues, its germline function is still unknown (Linder et al., 2008; Takei et al., 2021). To investigate TDRD-3's function in *C. elegans*, we generated multiple null alleles and GFP-tagged lines by CRISPR-Cas9. Super-resolution microscopy revealed its diffuse cytoplasmic expression and perinuclear foci in mitotic germline cells and embryo germline blastomeres, as well as colocalization with P granule markers PGL-1 and CGH-1. Under heat shock, TDRD-3 forms large cytoplasmic granules all around the germline and embryos, colocalizing with the dual GG and SG marker CGH-1.

Additionally, *tdrd-3* null mutants exhibit a minor brood size reduction at elevated temperatures. Based on these findings, we hypothesize that TDRD-3 regulates mRNA post-transcriptionally in granules and germline development. Our study aims to elucidate its role in mRNA regulation across GG and SGs, contributing to understanding TDRD3 function in granule organization in higher organisms. We will present our ongoing investigations and additional findings.

381C The *pos-1* 3□ untranslated region governs POS-1 localization and contributes to germline development in *C. elegans*

Haik V Varderesian[1], Beverly Ramirez[2], Melina Velcani[2], Juliet Utaegbulam[3], Sean Ryder[11]Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, [2]Worcester Technical High School, [3]Tufts University

Maternal mRNAs dictate cell fate specification during early embryogenesis. In *Caenorhabditis elegans*, a suite of RNA-binding proteins (RBPs) control when and where maternal mRNAs are translated into protein. POS-1 is a CCCH-type tandem zinc finger RBP required for posterior development. POS-1 represses *glp-1* mRNA translation in posterior cells of the early embryo. POS-1 binds directly to the 3 -untranslated region (UTR) of *glp-1* mRNA to regulate its translation. POS-1 binds to a sequence motif found in many maternal 3 UTRs, including in its own. The role of the *pos-1* 3 UTR has not been studied. We set out to describe how the *pos-1*3 UTR contributes to POS-1 patterning and reproductive fecundity. We show that the *pos-1* 3 UTR is essential for the POS-1 protein expression pattern. In wild-type worms, POS-1 expression begins in the one cell embryo after fertilization. In a *pos-1* 3 UTR deletion mutant, strong POS-1 expression is observed throughout the maternal germline. POS-1 expression in embryos is increased relative to control worms, but the pattern of POS-1 accumulation remains unchanged. Additionally, *pos-1* 3 UTR deletion mutant worms have a pleiotropic germline phenotype where a portion of adult progeny lack either one or both gonads. In addition, a subset of progeny that lack both gonads also display a multi-vulva phenotype. Lastly, all the phenotypes observed become more penetrant at slightly elevated temperature. Our results show that the *pos-1* 3 UTR contributes to maximum fecundity but is not essential for reproduction. Moreover, they show that the 3 UTR contributes to reproductive robustness during mild temperature stress.

382C Conditional GLD-2 depletion in the adult *C. elegans* germline reveals novel dose-dependent functions at different stages of the oocyte-to-embryo transition

Danni Li[1], Marina Winter[1], Rima Sakhawala[2], Graciela Galvez[2,3], Katherine McJunkin[2], Karl-Frederic Vieux[11]Biology &Biotechnology, WPI, [2]LCDB, NIH/NIDDK, [3]Biology, Colgate University

RNA translation and stability are critical to reproduction and development. Tailing is the addition of nucleotides to the 3′ end of RNA molecules in an untemplated manner. It is mediated by terminal nucleotidyl transferases (TNTs) and modulates RNA translation and stability. Historically, long poly(A) tails have been associated with stable and highly translated transcripts. Poly(A) tails in the oocyte and early embryo are particularly dynamic, yet the full scope of their function and requirement in regulating gene expression in this context is limited. GLD-2 is a noncanonical poly(A) polymerase that specializes in cytoplasmic poly(A) tailing and is critical for meiotic resumption in the *C. elegans* germline. *gld-2* null mutants do not produce oocytes and are completely sterile. In this study, the auxin-inducible degron (AID) system is used for acute spatiotemporal control of *gld-2* expression to study its function during the oocyte-to-embryo transition (OET). Only the near-complete depletion of GLD-2 (≥95%) in adult germlines (using RNAi + AID) phenocopies null mutants and completely upends meiotic entry and progression. In contrast, oocytes are continuously observed at the proximal end of gonads after AID-mediated GLD-2 depletion in adults (~90%). A consistent pattern of staggered and stacked oocytes does, however, point to defective terminal oogenesis in the proximal germline of AID mutants. DAPI-stained germlines exhibit normal meiotic entry and progression to diakinesis, and rule out meiotic defects – functionally dissociating meiosis from terminal oogenesis. The resulting brood displays a fully penetrant embryonic lethality phenotype, characterized by eggshell and cytokinetic defects, and chromosome de-condensation. Crosses with wild-type males did not rescue oogenesis defects or embryonic lethality. This comprises an OET defect that also implicates GLD-2 in terminal oogenesis and in early embryogenesis. Moreover, these results reveal different requirements for GLD-2 in different compartments of the *C. elegans* germline: a low, basal expression in the distal gonad, required for meiotic entry and resumption; and a more robust expression, required for viable oocyte production in the proximal gonad. Further characterization of AID mutants, using oogenesis markers (MEX-3, OMA-1, SPN-4), will resolve the molecular defects in oocytes, and Nano3P-seq, a direct cDNA sequencing method, will identify functional targets of GLD-2. This work seeks to identify critical genetic pathways and shed light on the central role of TNTs in regulating gene expression during the oocyte-to-embryo transition in *C. elegans*.

383C Insulin signaling during the second half of embryogenesis is required for developmental progression

Sydney S Vogel, Erin DiSandro, Bruce Wightman Muhlenberg College

Insulin signaling is essential for the progression of development in *C. elegans*. Mutations in the transcription factors *fax-1* or *unc-42*, which are essential for interneuron differentiation, result in decreased insulin signaling. As a result, *fax-1* or *unc-42* mutants, combined with a mutation in the insulin-like receptor *daf-2*, display a novel arrest phenotype at the end of embryogenesis referred to as perihatching arrest. This arrest state is similar to arrest in response to high salt concentrations, indicating that an osmotic stress pathway acts in opposition to the insulin-driven developmental pathway. We investigated the critical period for entering perihatching arrest by performing a temperature-shift experiment, and found that arrest depends on decreased insulin signaling between 8 and 10 hours (comma to three-fold stages of embryogenesis). This evidence, along with a decline in embryonic activity over a similar period, argues against a hypothesis in which insulin signaling evaluates developmental progression at the time of hatching.

We also evaluated the consequences of perihatching arrest. Rescue from perihatching arrest at 15oC is variable, taking between 4 and 7 days, which suggests that arrested animals may develop slightly slower than animals that have not experienced perihatching arrest. We are also examining the metabolic and health consequences of perihatching arrest by evaluating the lifespan of arrested animals.

384C A panoramic view of expression and function of the diverse and expanded Doublesex/Mab-3/DMRT gene family in *C. elegans*

Chen Wang[1], Yehuda Salzberg[2], Kristi Zoga[3], Meital Oren-Suissa[2], Michael P Hart[3], Oliver Hobert[11]Biological Sciences, Columbia University, [2]Weizmann Institute of Science, [3]University of Pennsylvania

Sexual differentiation in the animal kingdom is governed by a remarkably diverse array of regulatory factors. The only consistently used molecular players—albeit often at different stages of sex determination and differentiation—are members of the Doublesex/Mab-3 related transcription factor (DMRT) family. While these transcription factors have independently diversified in different species, no organism has been comprehensively examined for the complete repertoire of DMRT genes throughout all tissues and developmental stages. Consequently, the extent to which this gene family contributes to sexual differentiation remains unclear at the organismal level.

Using genome-engineered reporter alleles, we analyzed the expression of all 10 *C. elegans* DMRT genes in all cells and developmental stages in both hermaphrodites and males. Six exhibit sexually dimorphic expression in somatic and/or gonadal tissues. Within the nervous system, DMRT genes are expressed in 70% sex-specific neurons, and, strikingly, in 48% sex-shared neurons in a sexually dimorphic manner. These dimorphisms primarily emerge upon sexual maturation, with a few exceptions in early larval stages.

Functional analyses with genetically engineered null alleles revealed that the DMRT genes play distinct, highly cell type-specific roles in regulating male-specific and sexually dimorphic neuronal identities. Notably, one DMRT gene is male-specifically expressed throughout the sex-shared motor system, and its loss results in extensive feminization of locomotory behavior. Together, our study provides the first organism- and nervous system-wide view of the expression and function of this highly evolvable family of transcription factors.

385C Identification of *E. coli* SodB iron superoxide dismutase as a regulator of DAF-7/TGFβ "neuron-to-niche" signaling

Katherine X. Norton[1], Sophia M. Wasel[2], E. Jane Albert Hubbard[11]NYU Grossman School of Medicine, [2]Cell Biology, NYU Grossman School of Medicine

Stem cell behavior can be influenced by an organism's diet and sensory environment. In the *C. elegans* germline stem cell system, several nutrient sensitive pathways control the germline stem cell pool, one of which is the DAF-7/TGFß pathway. Our lab previously found that in favorable environmental conditions, ASI-expressed DAF-7 activates the relevant TGFß receptor (DAF-1) on the distal tip cell (DTC), the germline stem cell niche. In the DTC, receptor activity interferes with a repressor SMAD complex (DAF-3/DAF-5) to promote robust expression of the DSL ligand LAG-2, that activates Notch signaling in the neighboring germ cells. Because Notch signaling promotes the stem cell fate, favorable conditions thereby yield a larger germline stem cell pool (Dalfó et al., 2012; Pekar et al., 2017).

It has yet to be elucidated what dietary components regulate this "neuron-to-niche" signaling pathway. Taking advantage of the genetic tractability of *E. coli* as the worm's food source, we screened the *E. coli* Keio library of 3,985 nonessential *E. coli* gene knockouts. We screened the library in 96-well liquid format, assaying DTC expression of a *lag-2* promoter-driven reporter that is sensitive to DAF-7/TGF-β pathway perturbations. Image analysis with automated worm and DTC detection increased the overall throughput.

Our screen identified *sodB*, which encodes an iron superoxide dismutase. Superoxide dismutases reduce oxidative stress by converting superoxide into hydrogen peroxide. *C. elegans* worms raised on Δ*sodB E. coli* displayed diminished *lag-2* DTC reporter expression. After validating the mutant by DNA sequence analysis of the *sodB* locus, we demonstrated that plasmid-borne *sodB*(+) in the Δ*sodB* mutant restores *lag-2* reporter expression. Our results also implicate the DAF-7 pathway: *lag-2* reporter expression in *daf-3* null mutants was unaffected by Δ*sodB E.coli*, remaining at levels similar to wild-type worms raised on wild-type bacteria. Preliminary results from bacterial mixing experiments indicate that wild-type *E. coli* cannot compensate for Δ*sodB E. coli* suggesting that Δ*sodB* produces a toxic factor. We are testing the hypothesis that sulfites signal through DAF-7, altering *lag-2* expression in the stem cell niche.

386C It takes a crowd to keep FBF in check: multiple E3 ligases regulate FBF accumulation in *C. elegans* germline

Gabriella Weiss, Ekaterina Voronina University of Montana

PUF-family RNA-binding proteins, FBF-1 and FBF-2, control *C. elegans* germline stem cell maintenance through post-transcriptional gene regulation; however, the mechanisms regulating FBF protein stability are relatively understudied (Crittenden et al., 2002; Zhang et al., 1997). We identified an FBF-2 peptide (variable region 4, VR4) that was sufficient to induce degradation when fused to GFP and determined that its downregulation was dependent on the ubiquitin proteasome system. We hypothesized that FBF-2's VR4 functions as a degron recognized by specific E3 ubiquitin ligases.

An RNAi screen of predicted E3 ubiquitin ligases identified thirteen candidates that downregulated the GFP reporter. Further investigation revealed that most of these candidate E3 ligases influenced the stability of both GFP-tagged FBF-1 and FBF-2 transgenes, as well as an endogenously-tagged V5::FBF-2. Unexpectedly, none of them strongly affected the transition to meiosis, but instead, all affected the SPC region, which suggests that these predicted E3 ubiquitin ligases may function redundantly at the transition to meiosis. Since VR4 sequence is unique to FBF-2, the E3 ligases might recognize additional degrons in FBF-1 and/or function indirectly.

We also found that FBF-2's VR4 is necessary for degradation mediated by some, but not all, of the candidate predicted E3 ubiquitin ligases, suggesting additional degrons or indirect mechanism of action. Further investigation determined the addition of the FBF-2 VR4 peptide to FBF-1 did not change the max level of the chimeric protein in the mitotic cells, but accelerated its clearance at the transition to meiosis, which depended on the E3 ubiquitin ligases that differentially impacted the stability of wild type FBF-1 and FBF-2.

We also tested whether the E3 ligases independent of VR4 contribute to FBF destabilization observed in *csn-5* mutant background, which results in an oogenesis defect. A knockdown of three E3 ubiquitin ligases rescued the loss of oogenesis in *csn-5*, suggesting these E3 ligases may function within specific contexts in the germline. Overall, this work uncovers a complex network regulating FBF protein stability using VR4 and potentially other degrons to regulate FBF abundance within specific contexts, and thereby germline stem cell function.

387C Embryonic development of *C. elegans* sense organs

Leland Wexler[1], Irina Kolotuev[2], Maxwell Heiman[31]Boston Children's Hospital, [2]University of Lausanne, [3]Harvard Medical School

The sense organs in the head of *C. elegans* provide a model for understanding how different cell types interact to assemble a functional organ. Each sense organ is composed of two glial cells, called the sheath and socket, and one or more neurons. The sheath glial cell forms a seamless unicellular tube around the ciliated dendrite endings of its partner neurons. The socket glial cell forms junctions to itself, the sheath glial cell, and the hypodermis to create a tube-shaped channel through which the neuronal cilia protrude. A major challenge of studying sense organ development is the lack of tools available to directly observe the formation of these structures in the embryo. We mined a recently published high-resolution ultrastructural dataset obtained by focused ion beam scanning electron microscopy (FIB-SEM) of a comma-stage embryo for which fluorescence-based cell lineage tracing had provisionally annotated individual cells (Santella et al., eLife 2022). This dataset provides an untapped resource for visualizing early developmental events. From this dataset we reconstructed all the sense organs in the head including the two amphid (AM), four cephalic (CEP), six inner labial (IL), four outer labial quadrant (OLQ), and two outer labial lateral (OLL) sense organs. We found that many of these symmetric sense organs were at different stages of morphogenesis in this embryo, allowing us to infer the developmental steps by which they form. We found that, in many cases, sheath glia begin wrapping the progenitors of their partner neurons prior to their terminal division. Sheath wrapping occurs in an ordered process: the sheath glial cell begins wrapping its partner neurons at the distal tip of the dendrites, self-fusing into a tube as it completes its wrap, and then "zippering" down the dendrite. After sheath wrapping has begun, the socket glia begins circumferentially wrapping the sheath glia before presumably "telescoping" to form the mature channel. Surprisingly, we also observed two transient neuron-glia interactions that were previously undescribed: between the URY neuron and the OLQ sheath glial cell and between the AUA neuron and the amphid sheath glial cell. Our work demonstrates the value of large public EM datasets that can be repeatedly mined for new insights, and sheds light on how neurons and glia undergo their highly coordinated morphogenesis.

388C Lineage-Specific Developmental Variation in Wild *C. elegans* Isolates

Charlotte E Weymer[1], Ryan L Johnson[2], Erik Anderson[3], Pavak Shah[21]Integrative Biology and Physiology, UCLA, [2]Molecular, Cell, and Developmental Biology, UCLA, [3]Biology, Johns Hopkins University

Here we apply embGAN, a machine learning based pipeline for automating cell lineage tracing in label-free timelapse imaging of embryogenesis, to analyze developmental variation in a panel of wild C. elegans isolates. Our previous work showed lineage-specific variation in the distribution of cell cycle times between N2 and JIM113, a commonly used transgenic strain for automated lineage tracing. We expand this analysis to include genetically divergent strains from a range of geographic origins: CB4856, DL238, ECA36, JU2001, MY23, and XZ1516. Comparison of cell cycle distributions using the branch distance, a graph metric we previously developed for comparing lineage-aligned quantitative traits, shows approximately 4 clusters of embryos with most strains (N2, JIM113, CB4856 DL238, JU2001, and MY23) exhibiting often statistically significant, but qualitatively little strain-to-strain variation. Interestingly, ECA36 and XZ1516 show a high degree of divergence in lineage-specific patterns of cell cycle timing from the other strains with XZ1516 also showing similarly high degree of intrastrain embryo-to-embryo variation, especially in the posterior lineages of the embryo. We will present these observations alongside new applications of the branch distance to describe variation in cell positioning within the embryo. These results begin to define the range of phenotypic heterogeneity in C. elegans embryogenesis across wild genotypes.

389C Identifying factors that regulate Gli activation through genetic suppressor screens

Sanese K White-Brown, Yongquan Shen, Ronald Ellis Rowan-Virtua SOM

Gli proteins are conserved transcription effectors that can respond to the Hedgehog (Hh) signaling pathway; they control various organ and tissue developmental processes. Errors in Gli regulation can cause birth defects, developmental disorders, and cancers. Most Gli proteins undergo cleavage to form transcriptional repressors or remain uncleaved to work as transcriptional activators. Furthermore, each Gli isoform will need co-factors to perform these functions. We are studying the sole Gli protein in *Caenorhabditis* nematodes, TRA-1, to understand Gli protein regulation and function. Like other Gli proteins, TRA-1 has five highly conserved zinc fingers and is cleaved to produce a repressor of male genes. Recently, our lab showed that it also makes a full-length activator of the sperm genes *fog-1* and *fog-3*. Because nematode TRA-1 regulates only sex determination and reproduction, many assays for gene function are simple. Furthermore, nematodes lack classical Hedgehog signaling, so studying TRA-1 should aid in characterizing non-Hedgehog modes of regulation. Our key tool, the *C. briggsae tra-1(v48)* mutation, is in the activator domain, in which cleavage removes to form the repressor. This mutation prevents spermatogenesis in hermaphrodites, transforming them into true females, but does not affect somatic development. Thus, by analyzing the effects of *v48* on germ cells, we can study opposing TRA-1 activator and repressor functions and the genes that help conduct these activities. To help do this, I am screening for and characterizing suppressors of *tra-1(v48)* that restore hermaphrodite spermatogenesis. After screening over seven hundred genomes for dominant mutations, we have discovered two *v48* suppressors. Both are dominant for suppression but recessive lethal, suggesting they could identify essential genes that work with TRA-1. I have backcrossed the mutations and will begin mapping soon. In addition, we are setting up a second screen for mutations that mimic *tra-1(v48)* effects.

390C *tab-1/Bsx* regulates neuroglial and mesodermal lineage and fate specification

William B. Wilkerson, William Marchese, Alexis Garcia, Ella Eseigbe, Elicia Preston, John I. Murray Department of Genetics, University of Pennsylvania, Perelman School of Medicine

The invariant lineage and powerful single-cell gene expression resources make *C. elegans* embryogenesis an ideal system for identifying lineage regulators by reverse genetics. The ABala lineage adopts largely neuronal and glial fates, but transcription factors that specify ABala sublineages remain poorly understood. We identified several TFs expressed in ABala sublineages and screened mutants for lineage phenotypes using 4D confocal microscopy and automated cell lineage tracing. We focused on , which is primarily expressed in the ABalaaa lineage, for further study. TAB-1 is orthologous to the mammalian brain specific homeobox (BSX) gene, which is important for the development of the hypothalamus. Loss of *tab-1* causes the ABalaaa lineage to adopt a pattern of cell division and death consistent with a homeotic transformation to an ABalaap like fate.

To corroborate the functional role of *tab-1* in specifying terminal fates, we employed a series of fate-specific reporters in *tab-1* mutants. Previous work (Reilly et al 2022) found the loss of eight ABala-derived terminal neurons in *tab-1* mutants. We found that the ABala-derived ILso (lateral) glia are also lost, while the dorsal and ventral ILso, derived from non-*tab-1*-expressing lineages are unaffected. Outside of ABala, *tab-1* is expressed in laterally asymmetric patterns in the mother of the RIS and I5 neurons, and the MSpapp lineage. A pharyngeal valve reporter showed one or two additional valve cells in 78% and 11% of mutants respectively, suggesting an MSpapp to MSaapp fate transformation.

To investigate the regulation of *tab-1* expression, we mined the upstream regulatory region of *tab-1* for candidate enhancers using publicly available ChIP-seq and ATAC-seq datasets. Using enhancer reporter assays, we identified two modular enhancer regions, a proximal enhancer that appears to be sufficient to drive the ABala-lineage *tab-1* expression pattern and a distal enhancer that can drive the remaining asymmetric expression. Lineage-resolved expression data and motif analysis suggest several potential upstream regulators of *tab-1*. RNAi depletion of one of these, the Otx homolog ceh-37, leads to partial loss of TAB-1::GFP expression, suggesting additional regulators are present. Ongoing experiments aim to better understand these factors and provide insight on the necessity of each enhancer in *tab-1* expression patterning.

391C Identification of novel therapeutic targets for the treatment of Mitochondrial Fatty Acid Synthesis Deficiency

Devia Williams, Kim Nguyen, Darrick Gates, Sara Nowinski, Nick Burton Metabolism, Van Andel Institute

Inborn errors of metabolism (IEMs) are a group of genetic disorders that are individually rare but collectively affect approximately 1 in 2500 live births. Nearly a quarter of all IEMs represent defects in mitochondrial function. For example, defects in the mitochondrial fatty acid synthesis (mtFAS) pathway cause a collection of neurological defects and developmental delays while mutations in the mitochondrial fatty acid oxidation (FAO) pathway cause hypoglycemia and muscle weakness. Here, we used CRISPR/Cas9 to generate human patient variants in both the mtFAS pathway gene *mcat-1* (*Mcat* in humans) and the mitochondrial FAO pathway gene *let-721* (*Etfdh* in humans). In characterizing these *C. elegans* models of human IEMs, we found that while wild-type animals can develop to adulthood at 100% oxygen, both *mcat-1* and *let-721* missense mutants exhibit a highly penetrant developmental arrest in response to hyperoxia. We further demonstrate that this inducible developmental arrest can be used to perform chemical mutagenesis screening for mutations that revert the defects observed in mutant animals and that allow animals to resume development to adulthood at 100% oxygen. To date we have identified and begun the mapping of 6 novel mutations that restore *mcat-1* mutant animals' ability to develop under hyperoxia conditions. In the long-term, we propose that these models will not only reveal new insights into how mitochondria use and produce fatty acids but will also identify new therapeutic targets for IEMs that currently have no treatment options.

392C The Ral small GTPase is essential for exocyst-mediated exocytosis and can enhance exocytosis in response to signaling

You Wu[1,2], Yuchen Zhang[3], Regina Strakhova[4], Matthew Smith[4], Christopher M. Counter[3], David J Reiner[1,21]Institute of Biosciences and Technology, Texas A&M Health Science Center, [2]College of Medicine, Texas A&M University, [3]Department of Pharmacology and Cancer Biology, Duke University School of Medicine, [4]Department of Pathology and Cell Biology, Université de Montréal

The Ras small GTPase is the most frequently mutated oncoprotein. The Ras>Raf>MEK>ERK and Ras>PI3K>PDK>AKT cascades are well studied and pharmacologically targeted. However, the Ras>RalGEF>Ral (Ras like) pathway remains poorly understood. The exocyst, a heterooctameric complex conserved from yeast to humans, facilitates direct exocytosis from the Golgi by tethering secretory vesicles to the plasma membrane. Although yeast lack Ral proteins, mammalian cell-based assays suggest Ral both regulates exocyst-mediated exocytosis and uses the exocyst as a signaling intermediary. This dual role of Ral — as both a signaling protein and a putative component of a vesicle trafficking complex — has confounded efforts to understand the intersection of Ral and the exocyst via conventional biochemical methods. To investigate these questions, we are leveraging *C. elegans* genetics.

Our findings *in vivo* indicate that Ral is an essential regulator of the exocyst. Using tagged endogenous RAL-1/Ral and exocyst components SEC-5/Sec5 and EXOC-8/Exo84, we demonstrated that Ral binds to, colocalizes with, and genetically interacts with the exocyst to support exocytosis and normal development. This animal model has enabled us to uncover three key insights into Ral>exocyst function *in vivo*:

1) GDP-bound Ral may be sufficient for exocyst-dependent exocytosis. This observation suggests that Ral regulation of the exocyst in wild-type animals operates independently of GDP/GTP cycling and upstream signaling.

2) Constitutively Active Ras and Ral can compensate for reduced exocyst function. Consequently, we hypothesize that Ras>RalGEF>Ral signaling enhances exocytosis in response to cell-cell communication, revealing a new function for Ras.

3) Signaling-deficient Ral mutants, which can still GTP-load and hence activate, uncouples Ral's signaling role from its function in exocytosis. These mutants may expose a novel mechanism of Ral signaling that is potentially conserved across small GTPases.

In conclusion, our work advances the understanding of Ras>RalGEF>Ral signaling, paving the way for deeper mechanistic insights into Ral-exocyst interactions, as well as the development of biomarkers and therapeutics for tumors with elevated Ral activity.

393C Structure-directed gain-of-function and RBD-selective loss-of-function mutations in the AGE-1/PI3K catalytic subunit

You Wu[1,2], Tam Duong[1], Neal Rasmussen[1], Kent Rossman[3], David Reiner[1,21]Institute of Biosciences and Technology, Texas A&M Health Science Center, [2]College of Medicine, Texas A&M University, [3]Department of Surgery, School of Medicine, University of North Carolina, Chapel Hill

Phosphotidylinositol-4,5-bisphospate 3-kinase (PI3K) is a key signal transduction protein in both Insulin/IGF Receptor signaling (IIS) and Ras proto-oncogenic pathways. PI3K can also harbor oncogenic mutations. The PI3K>PDK>AKT cascade regulates glucose uptake, metabolism, cell growth, survival, and plays a central role in controlling *C. elegans* lifespan. However, the interactions between LET-60/Ras and AGE-1/PI3K remain unclear.

To optimize AGE-1 tagging, we modeled LET-60/Ras, AGE-1, and the AAP-1/p55 regulatory domain of PI3K onto the crystal structure of the orthologous human complex. To minimize interference with the signaling complex and plasma membrane, we designed a 30-residue linker plus tag at the C-terminus. Using CRISPR/Cas9, we inserted fluorescent protein (FP) and epitope tag sequences into the *age-1/PI3K* locus. Western blotting confirmed protein expressed at the expected size. The tagged AGE-1 exhibited no functional defects in phenotypic assays and FP-tagged AGE-1 was ubiquitously expressed across all stages without specific subcellular localization.

To generate constitutively active AGE-1, we identified residues conserved between oncogenically mutated human PI3KCA and AGE-1, introducing an E630K mutation via CRISPR; protein stability was unchanged. The gain-of-function (gf) AGE-1 reduced lifespan, reversed the extended lifespan of a hypomorphic *daf-2/InsR* mutant, and increased 1° vulval fate induction, phenocopying previously isolated PDK-1(gf) and AKT-1(gf). These results confirm the successful generation of an activated AGE-1 variant.

To disrupt the LET-60/Ras activation of AGE-1, we compared PI3KCA and AGE-1 sequences. Due to poor conservation of the Ras binding domain (RBD), we analyzed structural similarities and introduced R303E and K304E mutations in AGE-1 RBD. These mutations did not alter protein stability but increased animal stature and reduced dauer formation in the *daf-2/InsR*(rf) background. To further probe the role of LET-60, we introduced a T35S mutation predicted to retain LIN-45/Raf activation while impairing AGE-1 activation. LET-60(T35S) did not affect lifespan or vulval fate but phenocopied AGE-1 RBD(rf) in reducing dauer formation. These findings suggest a previously unrecognized LET-60>AGE-1 signaling role in constraining animal stature.

Our study highlights the value of human structural analysis for targeted domain mutations in *C. elegans* orthologs and reveals a novel signaling interaction.

394A Impacts of wildfire exposure on food-seeking behaviors in nematodes in Southern Oregon

Nikki P Andrews, Arielle Halpern, Melissa LaBonty Southern Oregon University

Since time immemorial, the west coast of the United States has experienced wildfires. Climate change is broadening the areas impacted by wildfires and increasing the severity of wildfires. Many aspects of wildfire have been studied but little is known about how exposure to wildfire changes animal behavior in regards to food sources. The aim of this research is to utilize wildtype *C. elegans* and other species of wild-isolated nematodes to assess attraction and aversion behaviors in response to compounds extracted from fire-exposed and unexposed food sources. Use of a model organism such as *C. elegans* can quickly and efficiently shed light on animal adaptations to the effects of wildfires. We selected acorns as our food source and extracted volatile odorant compounds for use in chemotaxis assays. Wild-isolated nematodes were collected from multiple sites in Southern Oregon and identified using morphology and genetic sequencing. 8-10 genera of wild-isolated nematodes were assessed for viability in chemotaxis assays, including *Pristionchus pacificus* and *Oscheius tipulae*. We are now running comparison assays of *C. elegans* and wild isolates, exposing them to varying concentrations of odorant extracts to assess behaviors and identify optimal experimental conditions. We aim to determine whether locally isolated animals display behaviors that mirror *C. elegans* or demonstrate adaptations to their unique natural environment. This research can provide greater insight into animal adaptations that may result from climate change-driven increases in wildfires.

395A The impact of dauer diapause on population dynamics

Galayna Baur[1], Molly Ripper[1,2], Andrea Scharf[11]Biological Sciences, Missouri University of Science and Technology, [2]National Human Genome Research Institute, National Institute of Health

The dauer stage is essential for *C. elegans* populations to survive harsh conditions. Dauers are a diapause stage induced by high population density, food scarcity, or temperature stress. While many studies have focused on the molecular and physiological changes associated with the dauer stage, much less is known about how the intricate interplay between genetics and environmental factors shapes wild *C. elegans* population dynamics. Here, we use a laboratory population system with *C. elegans* and *E. coli* to study *C. elegans* population dynamics under fluctuating environmental conditions complemented with an agent-based model for fast simulations. In this system, we can focus on the impact of environmental factors including inter-individual interactions by using clonal populations. We take advantage of the various strains with mutations in the dauer-relevant pathways available and compare well fed populations with populations experiencing 10-to-20-day starvation periods. One challenge is tracking dauers in our populations and dissecting the factors driving the dauer decisions at various stages of the populations. Currently we mostly rely on SDS tests to distinguish dauers from other larval stages. Our goal is to understand the importance the dauer-decision timing for population survival.

396A Microbial evolution to stress enhances host resilience and lifespan through metabolic reprogramming

Ajay Bhat[1], Rebecca Cox[2], Janvi Desai[2], Nupur Das[2], Megan L Schaller[2], Angela Tuckowski[2], Yatrik Shah[2], Scott F Leiser-[21]Molecular and Integrative Physiology, University of Michigan, [2]University of Michigan

Aerobic organisms are constantly exposed to reactive oxygen species (ROS), either from external sources or their own metabolism. As organisms age, their ability to respond to ROS declines, leading to cellular damage and age-related disorders. However, organisms are not isolated; they coexist with microbes that can sense ROS and possibly adapt faster than their hosts. This raises the question: could microbial evolution under oxidative stress influence host resilience and lifespan? To explore this, we employed two approaches: (1) adaptive laboratory evolution to generate ROS-evolved bacterial strains, and (2) testing the *C. elegans* microbiome for naturally ROS-resistant bacteria. While many bacteria from the microbiome screen enhanced *C. elegans* ROS tolerance, most reduced their lifespan. In contrast, ROS-evolved bacteria (OP50PQE) enhance both stress tolerance and lifespan of worms, suggesting their evolutionary adaptations provide additional benefits to the host. Whole-genome sequencing of OP50PQE revealed a mutation in the iron-sulfur (Fe-S) cluster repressor. These bacteria enhance ROS tolerance through iron-driven activation of the PMK-1 (p38/MAPK) pathway in *C. elegans*, linking bacterial iron metabolism to host signaling and aging. Disrupted bacterial Fe-S biogenesis could alter TCA metabolites, impacting mitochondrial metabolism and worm lifespan. Consistent with this hypothesis, we observe significant changes in TCA intermediates in OP50PQE. A targeted screen of bacterial TCA cycle genes showed that deleting mdh (malate dehydrogenase) and acnB (aconitase) under oxidative stress increased bacterial stress tolerance. Remarkably, worms fed mdh mutants live longer and activated the p38/MAPK pathway. Overall, our results suggest that microbial metabolic changes during oxidative stress influence TCA cycle flux, modulating host stress response and lifespan. These findings highlight microbial metabolic reprogramming during stress-mediated evolution as a novel strategy to enhance host aging and stress resilience.

397A Applications for nuclear genetic markers discordant with the *C. briggsae* phylogeny

Sierra Cantu-Gutierrez, Joseph Ross Biology, California State University, Fresno

Like *C. elegans*, its congener *C. briggsae* is a cosmopolitan species found worldwide. Much research in *C. briggsae* leverages the substantial genetic diversity among its wild isolate populations to study questions in ecology and evolution. The phylogeographic structure of *C. briggsae* populations is quite different from *C. elegans*, in that isolates from both tropical circles of latitude form a distinct clade from populations found in both northern and southern temperate regions and from equatorial populations. This tripartite clade structure suggests that there might be fixed genetic differences that distinguish the clades. A subset of those differences could be responsible for latitudinal adaptations like temperature sensitivity. The goal of this project is to identify readily PCR-genotyped tropical circles of latitude alleles that are distinct from temperate alleles. We have experimentally validated both amplified fragment length polymorphism and restriction fragment length polymorphism (RLFP) genotyping assays on all six nuclear chromosomes and in the mitochondrial genome in a panel of multiple temperate and tropical circles of latitude populations, including the common mapping populations HK104 and AF16. Most AFLP assays function as predicted based on the nuclear genome sequences of HK104 and AF16 as representatives of the temperate and the tropical circles of latitude clades, respectively. However, we also find that some indels are not diagnostic of phylogenetic clade, producing AFLP assays in which it appears that incomplete lineage sorting has occurred. This effort supports investigations requiring the identification of inter-population hybrids, such as in genetic mapping, and assists scientists in selecting which temperate and tropical circles of latitude populations can most easily be distinguished genetically using rapid and inexpensive techniques that are ideal for genotyping a single animal. In particular, nuclear alleles discordant with published molecular phylogenies are essential markers for control experiments in which intra-clade hybrids must also be identified.

398A *E. coli* Nissle association with insect-parasitic nematode *Steinernema hermaphroditum*

Victoria Chen[1], John P Marken[2], Richard M Murray[2], Mengyi Cao[31]Department of Biological Engineering, Massachusetts Institute of Technology, [2]Division of Biology and Biological Engineering, California Institute of Technology, [3]Division of Biosphere Sciences and Engineering, Carnegie Institution for Science

Steinernema spp are soil-dwelling and insect-parasitic nematodes that associate with mutualistic symbiotic bacteria of *Xenorhabdus* spp. The symbiotic pair has previously been established as a powerful experimental model to study nematode-bacteria interactions. During the infective juvenile (IJ) stage, *Steinernema* nematodes package species-specific symbiotic bacteria in their anterior intestinal pockets (receptacles) and can survive in the soil for months as they seek insect prey. However, the mechanisms of how these nematodes associate with environmental microbes other than their *Xenorhabdus* symbionts is barely known. Here, we report a new mechanism of *E. coli* Nissle (EcN) association with the nematode *Steinernema hermaphroditum*. We show that EcN cells, but not other *E. coli* strains we tested, are enclosed and lysed in at least four pairs of coelomocytes, suggesting these immune cells respond to bacterial invasion. During the IJ stage of nematode development, EcN cells localize to posterior intestinal vacuoles and enter the inter-cuticular space, where they proliferate, aggregate, then lyse. Proteins from bacterial lysates are maintained in the nematode cuticle over eight weeks in non-sterile soil. These observations suggest sequential steps of EcN colonization in the host nematodes involving an immune response that is distinctive from interactions with mutualistic symbiont *Xenorhabdus griffiniae*. Because *E. coli* Nissle is a highly tractable strain that is broadly used as a powerhouse for genetic engineering, our work establishes a novel framework of nematode-bacteria interactions with potential applications in environmental bioengineering.

399A Determining the genetic basis that defines microsporidia host specificity in *Caenorhabditis briggsae and Caenorhabditis nigoni*

Carolyn Chen, Winnie Zhao, Aaron ReinkeUniversity of Toronto - St. George

Microsporidia, an especially large phylum of obligate eukaryotic intracellular parasites, are a unique system to discover universal features of host-pathogen interactions in animals. These parasites are the simplest eukaryotes and as a phylum have evolved the capabilities to infect virtually every type of animal. *Nematocida parisii*, a naturally occurring microsporidian species, infects *Caenorhabditis* nematodes in the wild. A previous study conducted by the Reinke lab has shown that different *Caenorhabditis* species exhibit different susceptibilities to *N. parisii* infection. When exposed to the same amount of pathogen, *C. briggsae* demonstrates high levels of *N. parisii* infection while its sister species *C. nigoni* demonstrates low levels of infection. Through my research project, I have revealed that *C. nigoni* can actively eliminate *N. parisii* infection and that older *C. nigoni* animals are susceptible to infection. Together these results suggest that *C. nigoni* possesses immunity that restricts infection by *N. parisii*. To understand the genetic basis for this *C. nigoni* immunity, I have been taking advantage of the ability of *C. briggsae* and *C. nigoni* to form fertile hybrids. I have demonstrated that the interspecies hybrid progeny of *C. briggsae* and *C. nigoni* can be infected by *N. parisii*. Using *C. nigoni* strains that contain introgressed regions of *C. briggsae* chromosomes, I have also shown that four *C. briggsae* chromosomal regions cause *C. nigoni* to be susceptible to infection. I am currently conducting experiments to narrow down these intervals to identify the causative genes.

400A Monte Carlo simulation of somatic twist in ancient marine worms

Adam Cheong[1], John Cheong[21]Proof School, [2]Makeshift Lab

A somatic twist was thought to occur during the invertebrate-to-vertebrate transition 550 million years ago, resulting in a dorsoventrally inverted body plan for all vertebrates leading to decussation in the corticospinal tracts (Kinsbourne, 2013). Our research explored the plausibility of an evolutionary pathway that connects ancient marine worms to the protochordate *Pikaia* (aka the *'first fish'*). But what force(s) could possibly drive the biomechanical tissues of ancient marine worms to perform a somatic twist?

To recreate evolutionary events inside a computer, our experimental design requires Monte Carlo simulation of feast and famine cycles. Our research purpose is to observe how the first digital *C. elegans* worm from the OpenWorm Project evolves *in silico* to become the first fish, whose fossil was initially thought to be that of a "big fat annelid worm" when first discovered in the Burgess Shale, with a dorsoventrally inverted body plan after a somatic twist when given bountiful food sources over evolutionary time. Our hypothesis that underlies the simulation: underwater buoyancy from generations of feeding drove the somatic twist of ancient marine worms.

We downloaded an open source worm model from the OpenWorm Project and installed the Sibernetic software, which provides visualization of worm locomotion driven by a physics engine that simulates the hydrodynamics interaction between the environment and the body of the worm. We gathered model parameters for biomechanical matter within an aquatic environment and adjusted them according to cycles of feast and famine, in order to evolve successive generations of marine worms. We modeled feast and famine cycles stochastically using a Markov chain to drive proportionate changes of parameters characterizing each ring of the marine worm: body mass and volume, thus density and buoyancy based on the Archimedes' Principle. Our approach of integrating Monte Carlo simulation of feast and famine cycles over evolutionary time with a hydrodynamics engine for worm locomotion is a novel aspect of our research.

Monte Carlo simulation shows great promise as a practical new approach to conducting evolutionary biology experiments *in silico* when combined with Sibernetic and OpenWorm. Our research work is ongoing and we would like to share our interim progress in various design aspects of evolutionary modeling for a digital worm as well as practical computational challenges encountered along the way. We hope to ultimately be able to provide *in silico* validation for Kinsbourne's somatic twist theory, supported in turn by our hypothesis that underwater buoyancy force drove somatic twist in marine worms during evolutionary times when food was bountiful.

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401A Unichromosomal nematode genomes shaped by an evolutionary history of parthenogenesis, telomere dysfunction, and chromosome rearrangements

George Chung[1], Lewis Stevens[2], Xiaoguang Dai[3], Scott Hickey[3], Karin Kiontke[1], Priyesh Rughani[3], Manuela R Kieninger[2], Joanna C Collins[2], Sissel Juul[3], Fabio Piano[1], David HA Fitch[1], Mark Blaxter[2], Kristin C Gunsalus[11]Dept of Biology, New York University, [2]Tree of Life, Wellcome Sanger Institute, [3]Oxford Nanopore Technologies

In contrast to their dioecious relatives, members of the parthenogenetic *Diploscapter* nematode genus harbor their entire genome within a single pair of highly heterozygous chromosomes. To examine how this unusual karyotype relates to the evolution of parthenogenesis, we generated chromosome-level assemblies for two species in this clade: *Diploscapter pachys* and *Diploscapter coronatus*. Sequence comparisons revealed that the two genomes are colinear across their entirety, and that multiple ancestral chromosome fusions and extensive genomic rearrangements preceded the divergence of these two species. The presence of shortened telomeres and extended subtelomeric repeats suggests that the fusions arose from long-term defects in telomere function in the lineage. Intriguingly, our analysis also identified an introgression event after the two species' divergence, suggesting that their parthenogenetic lifestyle may have been punctuated by rare gonochoristic and sexual reproduction. These findings shed new light on how telomere loss, chromatin architecture, and reproductive strategies interconnect in shaping chromosome evolution.

402A Genetic variation in xenobiotic metabolism influences chlorfenapyr resistance in *C. elegans*

Timothy A Crombie[1], Michaela Foley[1], Nicolas D Moya[2], Erik C. Andersen[21]Florida Institute of Technology, [2]Johns Hopkins University

Environmental toxicants pose significant risks to human health, yet the genetic differences influencing susceptibility remain poorly understood. Chlorfenapyr, a widely used insecticide, has been restricted in some regions due to its persistence in the environment and toxicity to non-target organisms. However, its effectiveness in controlling malaria-transmitting mosquitoes has led to increased deployment in public health interventions. Understanding the genetic factors influencing chlorfenapyr susceptibility is crucial for assessing human health risks and the broader environmental impacts of the compound. Here, we leveraged the genetic diversity of *Caenorhabditis elegans* to investigate natural variation in chlorfenapyr susceptibility. Using a high-throughput phenotyping assay, we screened 152 wild *C. elegans* strains and identified a single outlier, ECA36, which exhibited extreme resistance. To map the genetic basis of this resistance, we generated recombinant inbred lines (RILs) from ECA36 and an intermediate-susceptibility strain, CB4856. Linkage mapping identified a major quantitative trait locus (QTL) on chromosome V explaining ~88% of phenotypic variance, overlapping a cluster of cytochrome P450 (CYP) genes involved in xenobiotic metabolism. Further investigation using long-read genome assemblies of 15 wild isolates revealed high variability in the QTL region, enriched for hyper-divergent regions (HDRs). Structural variation analysis identified two CYP gene variants unique to ECA36: a duplication of *cyp-34A9* and a deletion of *cyp-35A3*. Given the role of CYP genes in detoxification and the strong correlation between these structural variants and ECA36 resistance, we propose that these variants contribute to ECA36's chlorfenapyr resistance. Our findings highlight the power of *C. elegans* natural variation to uncover genetic determinants of toxicant susceptibility and underscore its potential as a model for assessing genetic contributions to toxicant responses in diverse populations.

403A Natural variation in *C. elegans* aggregation behavior reveals possible adaptation to changing environments

Youn Jae Kang[1,2], Antonio C Costa[3], Serena Ding[1,41]Max Planck Institute of Animal Behavior, [2]International Max Planck Research School (IMPRS) for Quantitative Behavior, Ecology & Evolution, [3]Sorbonne University, Paris Brain Institute (ICM), [4]Centre for the Advanced Study of Collective Behaviour

Extensive past research has focused on unraveling the genetic and molecular basis underlying the *C. elegans* aggregation phenomenon. However, detailed quantification of the natural variation of this behavior in wild populations, and whether this variation is shaped by adaptation to the local environment is yet to be explored.

We obtained 13 genetically divergent wild strains of *C. elegans* and their genomic information from the *Caenorhabditis* Natural Diversity Resource center to investigate natural variation in their aggregation behavior. Using quantitative measurements of aggregation from fluorescence recordings, we captured natural variation in both the magnitude and the timescales of aggregation behavior among the 13 strains. Accounting for relatedness among the strains, a linear mixed model reveals that this phenotypic variation is strongly explained by genetic variation, suggesting that both traits are heritable. Moreover, categorization based on climate information from the site of isolation revealed that strains from temperate regions show a consistent pattern of lower magnitude and shorter timescales of the behavior compared to those from the tropic/subtropic regions. Finally, the direction of genetic covariance aligns with the direction of phenotypic divergence between climate regions, possibly reflecting a behavioral adaptation to recently colonized temperate environments.

Taken together, our results suggest that aggregation behavior in *C. elegans* is an evolvable trait, which could have been shaped by the local environment that the strains have adapted to. This result could introduce a new perspective on *C. elegans* as a model system for the study of behavioral evolution.

404A Natural variation reveals hidden divergence in the evolution of a polyphenism

Stephen M Dreyer, Erik J Ragsdale EEB, Indiana University

Polyphenism, the ability of an organism to produce discrete alternative phenotypes in response to environmental conditions, exemplifies the non-linear relationship among genes, the traits they influence, and the environment. While several genes that regulate polyphenism have been identified in some systems, such as the nematode *Pristionchus pacificus*, which exhibits a resource polyphenism in the development of alternative mouth morphologies, it is mostly unknown which loci harbor intraspecific variation for polyphenism. To identify cryptic loci associated with polyphenism and natural evolutionary divergence, we created a panel of recombinant inbred lines derived from *P. pacificus* isolates that exhibit similar morph-bias ratios under common laboratory conditions. We found that the resulting recombinants included morph-bias phenotypes outside the parental range, indicating cryptic divergence between strains. Quantitative trait loci (QTL) analysis subsequently revealed three loci of large effect that influence morph production: two on Chromosome I, and one on Chromosome X that is epistatic over the autosomal loci. The X-linked QTL overlaps with a region known to contain the polyphenism switch gene *eud-1* and is consistent with recent work that similarly found this region to harbor variation for the polyphenism. However, functional validation by CRISPR/Cas9-driven allelic replacement revealed that the causal allele in our panel is different from that previously identified, indicating that this QTL has been targeted independently by evolution among different isolates of *P. pacificus*. Using CRISPR/Cas9-directed recombination in a near-isogenic line, we have developed a panel of additional recombinants within this region to fine map the causal locus to a 70-kb interval that excludes the gene body and known regulatory regions of *eud-1*. In summary, we describe a diverging polyphenism's genetic architecture, which consists of multiple QTL that together hide genetic variation by epistasis, and we show that a narrow, X-linked locus has been a convergent target of polyphenism evolution.

405A Environmentally acquired gut bacteria protect *C. elegans* from the toxic pesticide Chlorpyrifos.

Sarah El Khoury, Dan Kim, Shalini Aggarwal, Michael Shapira University of California, Berkeley

Animal gut microbiomes play crucial roles in host health and fitness, influencing various functions and possibly affecting evolutionary trajectories. Understanding the dynamics of host-microbiome interactions, particularly regarding the role of gut bacteria in environmental adaptation, is of growing interest in evolutionary biology, as microbiomes encode many more functions than the host genome and have the potential to respond to environmental stress rapidly and in diverse ways. *Caenorhabditis elegans* is a valuable model for microbiome research, harbouring a characteristic gut microbiome with persistent bacteria that contribute to host health and fitness. Taking advantage of this model, we explored the potential of gut bacteria for aiding hosts in withstanding exposure to environmental toxins, which pose unprecedented stress to all animals, including humans, and against which the biochemical diversity of bacteria could be particularly useful. This project investigates the potential of the gut microbiome to protect worms from Chlorpyrifos (CPF), an organophosphate and neurotoxic insecticide. CPF has been suggested to be toxic to humans and has adverse neurobehavioral impacts in *C. elegans*. We used compost-derived microbial extracts treated with CPF to enrich for potentially metabolizing bacteria and demonstrated that environmentally acquired gut bacteria were capable of protecting worms from the adverse effects of CPF on *worm* development and survival. Four protective gut isolates were selected for further characterization of their protective abilities and their interactions with the worm alone and in the context of a community, demonstrating differences in both regards. While these results are still preliminary, they support a substantial role for gut bacteria in determining host ability to adapt to a changing environment.

406A Nervous system evolution in highly divergent nematodes

Joke Evenblij[1,2], Oliver Hobert[1]1Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, [2]Institut für Genetik, Technische Universität Braunschweig

I use the well-studied and very stereotypical/tightly regulated nervous system of *Caenorhabditis elegans* as a basis and compare it to other nematode species that diverged many million years ago. The entomopathogenic nematode *Steinernema hermaphroditum* diverged > 100 million years ago from the free-living soil nematode *C. elegans*. About 400 million years ago the free-living freshwater nematode *Plectus sambesii* diverged from *C. elegans*. Such diverse habitats and life-styles likely involved substantial adaptations in nervous system structure and functions. I hypothesize that such adaptations involve (a) changes in the phenotypes (i.e.) of individual neuronal cell types (e.g. morphology and/or signaling capacities) and (b) that such changes in cellular phenotype may be brought about by re-organization of combinational codes of transcription factors (terminal selectors) that specify neuronal phenotypes.

As an initial starting point to identify adaptive changes, I am using hybridization chain reaction (HCR) and antibody staining to visualize the GABAergic, serotonergic, glutamatergic and dopaminergic nervous systems of *S. hermaphroditum* and *P. sambesii.* So far, I have observed similarities as well as differences with *C. elegans.* For example, the GABAergic nervous system seems to be conserved in number and location of neurons among all three nematode species, while *S. hermaphroditum* and *P. sambesii* harbor an additional dopaminergic neuron pair in their tail. I am examining the regulatory features that lead to those differences in neurotransmitter expression among nematodes through the analysis of expression patterns of terminal selector homologs in *S. hermaphroditum* and *P. sambesii*. Thus, I developed a protocol for gene deletions via CRISPR/Cas9 in *S. hermaphroditum* and have begun to knock out terminal selectors. I will report how a recently generated deletion of the LIM homeobox gene *ceh-14*, a terminal selector for many cell types in the *C. elegans* tail, affects cellular identities in the tail of *S. hermaphroditum*.

We anticipate that our analyses might provide overarching themes on how evolutionary processes led to the adaptation of nervous systems to extremely different habitats.

407A Competitors matter: transmission upon death selects for a higher pathogen load but not always an increase in virulence

Najmussher Ghani[1,1], Carline Zanchi[1], Payal Barua[1], Christoph Hartwig[2], Thomas Degenkolb[3], Charlotte Rafaluk[1]1Institute of Biology, [2]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, [3]Institute for Insect Biotechnology

The contemporary idea of pathogen virulence evolution mainly stems from the trade-off hypothesis between transmission, virulence, and recovery rate. In spore-forming parasites, however, the growth rate is separated into two stages: vegetative growth, through which the host is exploited, and spore production, which is closely linked to transmission potential. This separation means that parasite growth may not be simply correlated with harm to the host. Furthermore, the fact that spores can persist in the environment has the potential to reduce or alter the costs of virulence by allowing parasites to sit and wait after hosts are killed. Multiple mathematical models aim to extend the trade-off hypothesis and understand the evolution of virulence in spore-forming parasites. Some of these models predict that the timing of transmission (from living vs dead hosts) and whether parasites are in single or multi-strain infections influence the trajectory of virulence evolution. These effects are also expected to be linked to toxin production, as burst killers often produce toxins to kill their hosts and transmit further. However, despite the substantial mathematical exploration of these questions, there are still only very few empirical tests. We experimentally test the impact of parasite life history and related trade-offs *in vitro* and *in vivo*. We carried out an evolution experiment to directly test this hypothesis by manipulating the point of parasite transmission (living vs dead) and using toxin-producing and non-producing strains and a mix of these two followed by metabolomic, genomic, and phenotypic analysis. We found that transmission by burst killing results in higher pathogen loads, but whether this results in increased virulence depends on the timing of toxin production in the starting strain and most critically whether the fungus is in a single strain or multi-strain infection.

408A Network properties constrain natural selection on gene expression in *Caenorhabditis elegans*

Tyler R Inskeep, Simon C Groen Nematology, University of California Riverside

Gene regulatory networks (GRNs) integrate genetic and environmental signals to coordinate complex phenotypes and evolve through a balance of selection and drift. Using publicly available datasets from *Caenorhabditis elegans*, we investigated the extent of natural selection on transcript abundance by linking population-scale variation in gene expression to fecundity, a key fitness component. While the expression of most genes covaried only weakly with fitness, which is typical for polygenic traits, we identified seven transcripts under significant directional selection. These included *nhr-114* and *feh-1,* implicating variation in nutrient-sensing and metabolic pathways as impacting fitness. Stronger directional selection on tissue-specific and older genes highlighted the germline and nervous system as focal points of adaptive change. Network position further constrained selection on gene expression; high-connectivity genes faced stronger stabilizing and directional selection, highlighting GRN architecture as a key factor in microevolutionary dynamics. The activity of transcription factors such as *zip-3*, which regulates mitochondrial stress responses, emerged as targets of selection, revealing potential links between energy homeostasis and fitness. Our findings demonstrate how GRNs mediate the interplay between selection and drift, shaping microevolutionary trajectories of gene expression and phenotypic diversity.

409A The role of *C. elegans*-microbiome interaction in environmental adaptation

Inga Hamerich[1], Carola Petersen[1], Karen Adair[2], Hanne Griem-Krey[1], Johannes Zimmermann[1], Brendan Bohannan[2,2], Hinrich Schulenburg[11]Kiel University, [2]University of Oregon

Host-associated microbiomes play a fundamental role in shaping adaptation, yet the extent to which hosts and microbiomes jointly drive evolutionary processes remains unclear. Given that microbiomes can change more rapidly than host genomes, they may facilitate rapid adaptation to novel environments. To investigate this, we developed a model system using *Caenorhabditis elegans* and its microbiome in a complex environment. Over multiple generations, we observed diverse adaptive trajectories, with fitness outcomes influenced by both, host genetics and microbiome composition. Further analysis revealed shifts in microbial communities and host gene expression linked to these evolutionary changes. Our findings provide experimental evidence that host-microbiome interactions shape adaptation. Ongoing work explores how these interactions contribute to environmental modification like niche construction and long-term adaptive dynamics.

410A Sensory behavior promotes proper migration, and population growth and distribution in a soil-fruit simulated natural habitat

Jong-In Hwang[1], Jong-min Park[1], Rocel Amor O Indong[1], Chanjin Park[2], Jungwoo Lee[2], HanUI Yoon[3], Jin I. Lee[31]Division of Biological Science and Technology, Yonsei University Mirae Campus, Wonju, [2]Division of Computer and Telecommunication Engineering, Yonsei University Mirae Campus, Wonju, [3]Yonsei University Mirae Campus, Wonju

Sensory behaviors in *C. elegans* such as chemotaxis and thermotaxis have been comprehensively studied, and genetic, molecular and circuitry underpinnings of these behaviors have been elegantly elucidated by the scientific community throughout the years. However, whether these behaviors have relevance to the worm in their native habitats, and to what extent these behaviors contribute to the reproductive fitness of worms in their natural ecology has not been investigated. To study nematode behavior ecology, our group has developed a soil-fruit natural habitat (SFNH) to simulate the *C. elegans* natural environment in rotting apple and soil in the laboratory (Indong et al, 2024). After placing *C. elegans* in the SFNH, we observe robust generational population growth over a 15-day observation period. We find that adult *C. elegans* migrate throughout the diverse 3D environment including within the rotting apple and deep soil layers. These migration patterns do not appear to be stochastic, but instead adult worms may be displaying somewhat biased patterns of migration over time in the SFNH. Using a weak *unc-29* levamisole receptor mutant in the SFNH, we demonstrate that proper locomotion is necessary for population growth in native habitats. We designed a 3D migration assay to test whether sensory ability is necessary for optimal migration patterns. Results indicate that *tax-4* mutant are compromised for optimal migration, and *osm-6* mutants are completely unable to migrate in 3D. Interestingly, in SFNH *tax-4* mutants display loss of biased patterns of migration observed in N2, and a corresponding decrease in reproductive fitness in natural habitats. Currently, we are developing assays to test N2 and *tax-4* mutant chemotaxis and migration in soil media. In order to more accurately and quantitatively assess behavior and population growth patterns in the SFNH, we are developing a 3D AI/ML-based model that can calculate population growth, oviposition location and migration behavior in the SFNH and compare predicted and observed data using the power of *C. elegans* genetics.

411B Natural Variation in Gut Microbiome Bacterial Colonization Across Wild Strains of *Caenorhabditis elegans*

Wade Ingersoll, Robert Luallen Biology, San Diego State University

Caenorhabditis elegans occupies a range of geographic locations throughout the world. This global distribution has led to significant genetic variation, resulting in over 1,700 distinct strains. Despite substantial differences across these strains, their gut microbiomes are likely comprised of similar microbes. Hosts often rely on commensal bacteria found in their microbiome to provide protection from infection by preventing or delaying pathogen niche formation. This presents a unique problem where *C. elegans* must balance its bacterial load to ensure sufficient protection without succumbing to infection. We discovered a *C. elegans* commensal-like microbiome bacterium, *Lelliottia jeotgali* (LUAb3), that attaches to the intestinal epithelium and completely colonizes the gut lumen of N2. Additionally, we have shown that this bacterium can protect against colonization by an adherent bacterial pathogen, named *Candidatus* Lumenectis limosiae. We hypothesize that genetic differences across *C. elegans* lead to natural variation in bacterial colonization. To test this hypothesis, we administered LUAb3 to fifteen wild strains of *C. elegans* and measured colonization using fluorescence *in situ* hybridization. Our results showed considerable variation in colonization rates across the fifteen strains. Importantly, computational analysis using the *Caenorhabditis* Natural Diversity Resource revealed that strains with extremely low colonization rates shared 1,319 common mutations, whereas highly colonized strains shared only 251. This supports our hypothesis that a greater number of mutations is associated with reduced colonization. Lastly, we recently identified five genes required for LUAb3 colonization via forward genetic screens, which we called *kola1-5.* Among the wild strains with extremely low colonization rates, we observed a positive correlation between the number of mutations in *kola1-5* and LUAb3 colonization; this effect was less pronounced in strains with WT-like colonization. Next, we will challenge these strains with pathogenic bacteria through lifespan experiments to determine whether low-colonization strains survive longer than high-colonization strains when challenged with *Ca* L. limosiae.

412B Interrogating the influence of *Xenorhabdus* symbiotic bacteria on the transcriptional landscape of *Steinernema* nematodes

Sally W Ireri, Mengyi Cao Carnegie Science

Steinernema spp are insect-parasitic nematodes that are emerging genetically tractable organisms useful in studying microbial symbiosis. During a free-living infective juvenile (IJ) stage, the nematodes house mutualistic Xenorhabdus bacteria in an intestinal pocket and together the symbiotic partners infect insects. Once in the insect cadaver, nematodes develop while feeding on their symbiotic bacteria. Nutrient depletion and overcrowding induces the formation and dispersal of IJs through the soil in search of a new insect host. Although the molecular mechanisms underlying the bacterial side of the mutualistic association are well characterized, the effects of symbiotic bacteria on Steinernema nematode physiology remain largely unexplored. In this study, we performed RNA-sequencing on the nematode S. carpocapsae to investigate how its symbiotic bacterium, Xenorhabdus nematophila, affects the transcriptome of host IJs. Our data show that more than 200 genes are differentially regulated in S. carpocapsae IJs that are colonized by symbiotic bacteria in comparison to their axenic (gnotobiotic) counterparts. Differentially expressed genes (DEGs) include homologues known in C. elegans to be involved in nematode immune response and metabolism. To explore if the top DEGs in S. carpocapsae IJs are conserved in another Steinernema - Xenorhabdus symbiotic pair, we performed digital PCR to test the gene expression patterns in a genetically tractable species, S. hermaphroditum, under both the axenic condition and when colonized by their symbiotic bacterium X. griffiniae. Out of six genes tested, three genes showed an expression pattern that are unique to S. carpocapsae and three are conserved in S. hermaphroditum. One of the genes with a conserved expression pattern, a homologue of hrg-1, encodes a heme-binding transmembrane transporter. We observed upregulation of its expression in the axenic condition in comparison to the symbiont-colonized conditions in both species which is an indication of heme starvation. This observation is unexpected as the axenic growth media should contain ample heme from beef liver and kidney which is believed to mimic the insect host environment. Our data implies that symbiotic bacteria are crucial for heme uptake in the Steinernema nematodes and may play a role in host iron metabolism. Overall, our data builds a framework to help uncover the molecular mechanisms through which symbiotic bacteria modulates its host's physiology.

413B Preparation of staged *Caenorhabditis elegans* embryos using size filtration

Nikita S Jhaveri, Maya K Mastronardo, J. B. Collins, Erik C Andersen Biology, Johns Hopkins University

Most *Caenorhabditis elegans* experiments require a specific developmental stage because of the distinct morphologies, behaviors, and gene expression patterns of each stage. In addition, high-throughput assays such as genome-wide association studies (GWAS), drug screens, proteomics, and behavioral assays require a large number of animals at a particular stage. To synchronize the *C. elegans* developmental stage, embryos are collected and incubated at the desired temperature until the animals grow to the target stage. The most common way of obtaining embryos is by dissolving the hypodermis of gravid adults with a hypochlorite (bleach) solution. However, different parameters, such as the bleaching time and temperature of the bleach solution, affect the survival of the embryos. Microfluidic devices have been used as alternatives, but they are expensive and require customization to scale up the preparation of many staged animals. Mesh filters of different sizes are a cheaper option to microfluidic devices and have been used by various groups for the physical separation of differently sized animals. However, the process is often difficult to perform because of the small differences in length and width between consecutive larval stages, making it difficult to get a completely pure culture of a specific stage. Here, we present a protocol for isolating embryo cultures using mesh filters. Once isolated, embryos are cultured to obtain a synchronous population of L1 larvae that can be grown to a synchronized stage-specific population. Using the filtration protocol, we obtained a higher yield of embryos per plate than using a bleach synchronization protocol and at a scale beyond microfluidic devices. We further showed that the embryos obtained by filtration developed into healthier larvae compared to the larvae obtained by bleach synchronization. In conclusion, we have exploited the differences in the sizes of *C. elegans* developmental stages to isolate embryo cultures that bypass the traditional method of bleach synchronization and are suitable for use in high-throughput assays. Our technique provides a robust way of collecting large numbers of *C. elegans* embryos with high purity without the need to subject the animals to harsh chemicals like bleach.

414B Imaging microbiome population dynamics

Svilen Kolev, Derick Ou, Vy Nguyen, Dylan Lasky, Sophia Cofone, Erel Levine Northeastern University

The composition and dynamics of the gut microbiome are shaped by complex interactions between environmental factors, host selection, and microbial competition. To study these interactions and their role in community assembly, we have developed an experimental platform using the model organism *C. elegans* and its tractable 10-species microbial community (CeMbio).

Our experimental platform employs microfluidics to individually confine hundreds of *C. elegans*, facilitating precise environmental control and longitudinal imaging. Using fluorescently labeled bacteria and hardware-enhanced widefield microscopy, we track gut microbiome dynamics over 20-hour periods. We automate image analysis to quantify microbiome load and spatial distribution.

In day 1 adults worms, we observe stochastic, nonequilibrium dynamics dominated by host selection. Most bacteria are transiently present, being rapidly digested or expelled; however, certain species can form persistent clusters. In day 3 adults, microbiome dynamics differ markedly. We observe significantly higher bacterial accumulation, progressive migration along the length of the gut, and substantially increased persistence. We test the strength of host selection on these dynamics using mutant strains and wild isolates. With age, host selection strength decreases, while historical composition becomes relevant to microbiome dynamics.

Our system facilitates detailed analysis of both short-term mechanically driven spatial arrangements and longer-term host-mediated interactions, establishing *C. elegans* as a valuable model for uncovering ecological principles governing microbiome assembly and stability.

415B The ecology of *C. elegans* generational population growth in a soil-fruit natural habitat setting in the laboratory

Jin I. Lee, Rocel Amor O. Indong, Jong-In Hwang, Jong-min Park, Chanjin Park, Jungwoo Lee, HanUl Yoon Yonsei University Mirae Campus, Wonju

All of us in the *C. elegans* community are well aware of how fast the worm develops, and how quickly worms lay eggs and can populate a plate. In nature, however, *C. elegans*, is found in soils of temperate environment seeded with rotting vegetation or fruit. In such a varied environmental setting, worm population growth patterns and ecological relationships are different and more complex than the laboratory. Our group has devised a method called the soil-fruit natural habitat (SFNH) to culture and study *C. elegans* in an environment closely simulating their natural ecology (Indong et al, 2024). Over the 15-day worm monitoring period, *C. elegans* displays a robust population growth peaking in the tens of thousands. However, still pales in comparison to optimal population growth with OP50 on NGM plates. Using mutant analysis, we find that *spe-11* mutants that carry sperm-specific fertility defects display overall similar trends in population growth decrease in NGM and SFNH culture over time. In contrast, we find *unc-29* mutants defective in movement and *osm-6* mutants defective in sensory are unable to populate the SFNH environment. We have evaluated the spatial distribution of the worms over time in the SFNH, and we find skewed population distribution trends suggesting adult worms migrate to specific places in the environment. Interestingly, we find that adults and L1 young larvae are often found in different areas of the SFNH suggesting that the adult worms lay eggs may be laying eggs in specific areas of the SFNH. In addition, we are overlaying the population dynamics data with microbial communities found in the SFNH over time. Finally, we are developing a 3D AI/ML-based model that can calculate population growth, oviposition location and migration behavior in the SFNH and compare predicted and observed data using the power of *C. elegans* genetics.

416B Isolation and genomic analysis of *Oscheius tipulae* and its associated microbiome from Yeonpyeongdo Island, Incheon, Korea

Daisy S Lim[1], Junho Lee[21]The Research Institute of Basic Sciences, Seoul National University, [2]Departement of Biological Sciences, Seoul National University

Studies of nematodes living in the wild environment of Korea are limited. Here, we report the isolation of a free-living Rhabditidae nematode, *Oscheius tipulae*, from soil samples collected from Marine Park in the coastal area of Yeonpyeongdo Island, located in the Yellow Sea near the Demilitarized Zone (DMZ). Whole-genome sequencing (WGS) analysis revealed a close phylogenetic relationship between this isolate and other wild isolates from East Asian regions of China and Vietnam, consistent with its geographic origin. Notably, a significant portion of WGS reads did not map to the *O. tipulae* reference genome, likely derived from the nematode›s associated gut microbiome; we did not attempt to remove the bacteria associated with the nematode to minimize the risk of malnutrition and mortality. Kraken2 analysis of unmapped reads indicated an enrichment of bacterial sequences, including those from *Brucella* and *Chryseobacterium* genera. This study provides insights into the genetic diversity and associated microbial communities of *Oscheius tipulae* in the Korean peninsula.

417B A fig-associated *Klebsiella* isolate reveals microbe-dependent life history evolution in *Caenorhabditis* nematodes

Austin C. Link, Gavin Woodruff School of Biological Sciences, University of Oklahoma

Animals live in a microbe-rich world, and host-microbe interactions influence fitness and organismal health in both beneficial and detrimental manners. *Caenorhabditis* nematodes have been a model system for half a century. While experimental studies with these nematodes have long been conducted with the human commensal *E. coli* as nematode food, only recently have studies been conducted with microbes isolated from relevant ecological contexts. How can natural microbial associates inform the biology of longstanding experimental systems? To understand the evolution of host-microbe interactions, we have isolated approximately one hundred strains of wild microbes associated with the nematode *C. inopinata* in nature. *C. inopinata* is the closest known relative of *C. elegans* and inhabits a divergent ecological niche while having distinct, divergent morphology. Here, we have reared *C. inopinata* and *C. elegans* on a subgroup of our bacterial isolates and have measured various life history traits. We found that a novel isolate, from the genus *Klebsiella*, doubled population growth for both *C. inopinata* and *C. elegans* compared to *E. coli*. However, fitness is modulated by divergent life history mechanisms in the two species. *Klebsiella* sp. WOUb2 increases individual fecundity in *C. elegans*, whereas it increases the developmental rate of *C. inopinata*. By sequencing fig suspensions, we determined this bacterial species is likely frequently found in the figs *C. inopinata* inhabits, although its relative abundance is not correlated with nematode presence. Rearing nematodes on this ecologically relevant isolate has revealed divergent life history trajectories of these animals, and future work will be done to investigate the molecular and genetic bases of these host-microbe interactions.

418B A gene encoding ubiquitin ligase adapter suppresses directional hybrid lethality between *Caenorhabditis briggsae* and *C. nigoni*

Yiqing Liu[1], Jeffrey, Dongying Xie[1], Tongshu Wen[1], Zhongying Zhao[21]Hong Kong Baptist University, [2]hong kong Baptist University

Speciation is driven by various forms of reproductive barrier, leading to hybrid incompatibility (HI) between populations or closely related species. Despite decades of research, the underlying molecular mechanisms of HI remain largely enigmatic. The key to understanding HI is to establish the molecular identity of the genes underlying various HIs between populations or closely related species. Nematodes *C. briggsae* and *C. nigoni* have emerged as a model for such study due to the partial incompatibility in their hybrid progeny. Numerous HI phenotypes have been uncovered between the two species, but specific HI genes that are molecularly cloned remain extremely rare. We previously identified two independent *C. nigoni* loci, *shls-1* (Species Hybrid Lethality Suppressor) and *shls-2*, that are indispensable for the viability of hybrid F1 embryos between the two nematodes. Here, we report the identification of a third hybrid suppressor allele, *shls-3*, which is responsible for parent-of-origin dependent hybrid lethality. By crossing *C. briggsae* and *C. nigoni*strains carrying a specific *C. briggsae* genomic introgression (referred to as "introgression stains" hereafter), we demonstrated the homozygosity of the left arm of *C. briggsae* Chr. II in the F1 hybrids leads to nearly 100% embryonic lethality when *C. briggsae*, but not *C. nigoni*, acts as father. Aided by CRISPR/Cas9-mediated targeted recombination, we narrowed the candidate interval for *shls-3* to approximately 90 kb through the systematic reduction of introgression size. We molecularly cloned the *C. nigoni* gene through systematic deletion of the candidate genes within such interval. *Cni-shls-3* encodes a putative ubiquitin ligase adaptor protein. Although this gene belongs to a family unique to *C. nigoni* and *C. briggsae*, we found that it is essential for the viability of both hybrid F1 and *C. nigoni*. We hypothesize that the gene's essentiality may be attributable to its role in a toxin-antidote system, and are actively working on the cloning of the toxin-encoding gene. The discovery of a novel HI gene provides invaluable insight into the mechanisms of reproductive isolation between the two species.

419B An empirical test of Baker's law: Dispersal favors increased rates of self-fertilization

Michelle McCauley[1], Levi Morran[21]Biology, Florida International University, [2]Emory University

Baker's law is the observation that recently dispersed populations are more likely to be self-fertilizing than populations at the range core. The explanatory hypothesis is that dispersal favors self-fertilization due to reproductive assurance. *Caenorhabditis elegans* nematodes reproduce via either self-fertilization or outcrossing and frequently disperse in small numbers to new bacterial food sources. While *C. elegans* males facilitate outcrossing, males and outcrossing are rare in natural *C. elegans* populations. Here, we use experimental evolution to test if frequent dispersal selects for the invasion of self-fertilization into predominantly outcrossing populations. *C. elegans* dispersal often occurs in the dauer alternative life stage. Therefore, we tested the effects of dispersal on rates of self-fertilization in populations exposed to dauer-inducing conditions and populations maintained under standard lab conditions. Overall, we found that populations required to disperse to new food sources rapidly evolved substantially elevated rates of self-fertilization compared to populations that were not required to disperse in both dauer and non-dauer populations. Our results demonstrate that frequent dispersal can readily favor the evolution of increased selfing rates in *C. elegans* populations, regardless of life stage. These data provide a potential mechanism to explain the dearth of outcrossing in natural populations of *C. elegans*.

420B Decoding microbiome-mediated modulation of *Pseudomonas aeruginosa* infection dynamics in *Caenorhabditis elegans*

Vy T Nguyen[1], Svilen Kolev[2], Erel Levine[1,21]Bioengineering, Northeastern University, [2]Chemical Engineering, Northeastern University

Pseudomonas aeruginosa is an opportunistic pathogen causing lethal infections in immunocompromised hosts, where microbiome dysbiosis is associated with increased severity and susceptibility. While this suggests microbiome-mediated protection, the spatial-temporal dynamics of *P. aeruginosa* infection in the presence of the microbiome remain poorly understood. This work aims to investigate the effect of microbiome composition on *P. aeruginosa* infection dynamics using the model organism *C. elegans*. We will leverage the transparent body plan and well-defined microbiome (CeMbio) of *C. elegans* to directly observe gut colonization dynamics of fluorescently labelled microbiome species and *P. aeruginosa* (PA14). We are in the process of leveraging microfluidics technology (HandKAchip and WormSpa) and fluorescent microscopy to conduct killing assays and to capture the spatial-temporal dynamics of PA14 in animals cultivated on CeMbio species. In addition, we intend to use genetic perturbations in immune response pathways to determine if the protective effect of microbiome colonization is due to induction of host immune response or a result of direct and antagonistic interactions between microbiome members and PA14. In our initial characterization, we observed that adult animals cultivated on a mixture of CeMbio species displayed enhanced survival once exposed to PA14. These results suggest that colonization by a complex microbiome offer a protective effect from pathogen infection which we hypothesize is due to decreased or delayed accumulation of PA14 in the gut. This research will advance our understanding of *C. elegans* microbiome interactions by elucidating mechanisms through which the microbiome affects PA14 infection. Our findings will provide critical insights into the fundamental principles governing microbiome-pathogen interactions and could potentially inform strategies for manipulating microbiomes to prevent pathogen infections, with implications extending to human health.

421B Pangenome gene-set analysis in *Caenorhabditis elegans*

Lance M. O'Connor, Nicolas D. Moya, Robyn E. Tanny, Erik C. Andersen Department of Biology, Johns Hopkins University

Caenorhabditis elegans primarily reproduces by self-fertilization, and evolutionary theory suggests that this mode of reproduction can lead to an "evolutionary dead end" because of reduced genetic diversity and long-term adaptation to heterogeneous environments. In previous studies, we amassed 1,631 wild *C. elegans* strains, sequenced the genomes using short-read technologies, and found that these strains contain punctuated genomic regions of extreme genetic diversity (hyper-divergent regions, HDRs) when compared to the reference N2 strain. These HDRs are enriched for genes associated with environmental responses such as xenobiotic stress and olfaction and might play a role in adaptation. However, the gene content of these regions remains incomplete because the extreme divergence in HDRs between the wild and reference genomes complicates short-read DNA alignments. To address this limitation, we generated de novo assemblies of 114 strains using PacBio HiFi long-read sequencing. Then, we exploited short-read RNA sequencing data to predict protein-coding genes in these newly assembled genomes and identified thousands of novel genes not found in the N2 reference strain. We classified both reference and novel genes as «core» (*i.e.*, found in >95% of all strains), "core-accessory" (*i.e.*, found in >5% but <95% of all strains), "rare" (*i.e.*, found in <5% of strains), or "unique" (*i.e.*, found in only a single strain). Additionally, we identified single-nucleotide and structural variants (*i.e.*, large deletions and insertions) that affect these new genes and genes found in the reference genome. This pangenome gene set will be used to create new genotype matrices and perform genome-wide association mappings to connect gene content differences to phenotypic variation across the species, further elucidating the molecular mechanisms of adaptation.

422B Genome-wide variant annotation in selfing *Caenorhabditis* species

Lance M. O'Connor, Michael E.G. Sauria, Nicolas D. Moya, Robyn E. Tanny, Erik C. AndersenDepartment of Biology, Johns Hopkins University

In the model organism *Caenorhabditis elegans* and related self-fertilizing (selfing) species *Caenorhabditis tropicalis* and *Caenorhabditis briggsae*, genetic variation has been detected among wild strains. This genetic variation is naturally occurring, making it difficult to assess whether specific variants have beneficial, deleterious, or neutral effects on organism fitness. Variant annotation tools aim to predict the effects of small variants and how they might perturb gene functions. Some variant annotation tools assess the effect of an isolated variant on a genomic feature, but others assess the effect of multiple variants found in the same haplotype, referred to as compound variants. Additionally, certain tools only annotate missense variants and provide quantitative scores based on the predicted deleteriousness of the amino acid change by assessing positional amino acid conservation from multiple sequence alignments. Given the unique or corroborative information individual variant annotation tools provide, we can integrate multiple tools to holistically assess the predicted consequence(s) of small variants. We have created an interactive database on the *Caenorhabditis* Natural Diversity Resource (CaeNDR) website to provide the community with a way to parse variants found genome-wide among strains of the three selfing *Caenorhabditis* species. According to evolutionary theory, deleterious variants are expected to be removed by purifying selection. Therefore, understanding the functional role of the naturally occurring variants detected among wild strains of selfing *Caenorhabditis* species might provide insights into how these androdioecious species maintain genetic variation.

423B Cardiac glycoside resistance in steinernematids and wild isolates of *C. elegans*

Robert A Pena, Perla Achi, Lanie Bavier, Cullen McCarthy, Anil Baniya, Preston Christensen, Victoria Iglesias, Simon C Groen, Adler R Dillman Nematology, University of California, Riverside

Cardiac glycosides (CGs) are toxic compounds produced or sequestered by certain plant and animal species for defense against enemies. CGs derive their toxicity to animals from inhibiting the Na+/K+-ATPase (NKA), a critical ion pump necessary for maintaining cell osmotic equilibrium and membrane potential. While some organisms that carry or otherwise encounter CGs evolved resistance through target site insensitivity (TSI) via substitutions in the NKA's alpha subunit, others appear to have evolved alternative resistance mechanisms. For instance, milkweed herbivores such as the monarch butterfly and one of their natural enemies, the parasitic nematode *Steinernema carpocapsae* exhibit TSI. However, the congener *Steinernema feltiae* shows partial CG resistance without some of the large-effect TSI substitutions, suggesting an alternative protective strategy. Likewise, some resistant insects, such as the milkweed tussock moth *Euchaetes egle*, lack TSI substitutions entirely. These observations point to the existence of possible additional CG resistance mechanisms such as toxin efflux via ABC transporters or metabolic detoxification. Our study aims to uncover these alternative resistance mechanisms through studying wild isolates of *Caenorhabditis elegans* in the *Caenorhabditis* Natural Diversity Resource (CaeNDR). By conducting toxicity assays with ouabain, a model CG, we have identified resistant *C. elegans* strains. We are applying genome-wide association studies (GWAS) to determine genes and genetic variants linked to CG resistance. Our findings will shed light on the genetic architecture underlying CG resistance and emphasize the need to explore resistance mechanisms beyond TSI. Our research has broader implications for chemical ecology, evolutionary biology, and drug resistance studies.

424B The role of bacterial biosynthetic gene clusters in microbiota-mediated protection

Lena Peters, Barbara Pees, Katja Dierking Kiel University

Protection against pathogens is a major function of the gut microbiota. Although bacterial natural product biosynthetic gene clusters (BGCs) have emerged as crucial components of host-microbiota interactions, knowledge on their function in microbiota-mediated protection is scarce. We use *C. elegans* as experimental system to study mechanisms of microbiota-mediated protection. Our current research focuses on the protection that two natural *Pseudomonas* isolates, *P. lurida* (MYb11) and *P. fluorescens* (MYb115), provide against the pathogen *Bacillus thuringiensis*. I will present findings on *C. elegans* microbiota BGC-derived molecules and their associated biological activities, and highlight the role of these molecules in microbiota-mediated protection.

425B Isolation and Characterization of Worm Attractants Secreted By Beneficial *Pantoea* Gut Commensals

Jeremy Pietropaolo, Michael Shapira Integrative Biology, UC Berkeley

C. elegans harbors a characteristic and persistent community of gut bacteria that contribute to worm physiology including its development, behavior, and infection resistance. Previous work in our lab revealed differential contributions of *Pantoea* species, in which gut isolates were good colonizers and increased resistance to a pathogen, while their environmental congenerics were significantly inferior in both. These studies further revealed that worms preferred Pantoea species that would be beneficial gut commensals over their non-beneficial environmental congenerics (Pérez-Carrascal et al., 2022). We have since shown that worms are attracted to a secreted signal that was partially volatile. Bioactivity-guided liquid chromatography fractionation and gas chromatography-coupled mass spectrometry identified two small molecules as potential attractants demonstrating dose dependence. Concomitantly with identification efforts of the small molecule attractants, experiments using *C. elegans* mutants suggested that the *odr-10* gene, encoding a G-protein coupled receptor expressed in AWA neurons, may be involved in sensing the attractants. Additional receptors in the AWA, AWB, AWC, and ASH neurons are being explored. Behavioral preferences of beneficial microbes is a yet underexplored aspect affecting microbiome composition. Unveiling the signals and underlying mechanisms will advance our understanding of the factors that shape microbiome composition and its contributions to host health and fitness.

426B Evolution of selfishness via recycling of ancestral protein interaction modules

Florian Puehringer[1], Polina Tikanova[2], Belén Pacheco-Fiallos[2], Gang Dong[3], Alejandro Burga[21]IMBA Vienna, IMBA Vienna, [2]IMBA Vienna, [3]MPL Vienna

Selfish genetic elements are pervasive and universal features of our genomes; however, we know remarkably little about their molecular mechanisms and evolution. This lack of knowledge is most obvious for eukaryotic toxin-antidote elements (TAs), the most extreme selfish elements identified to date – capable of killing progeny that do not inherit them. TAs consist of two linked genes: a toxin and its cognate antidote. The toxin is maternally deposited into unfertilized eggs irrespective of their genotype but only offspring that inherits the TA element can zygotically express the antidote and survive.

In this study, we investigated the molecular basis and evolutionary origin of the *C. elegans sup-35/pha-1* TA. The toxin, SUP-35, is a paralog of the microtubule-binding protein RMD-2 and it requires two host genes for its toxicity: *sup-36* and *sup-37*. However, the molecular basis of this tripartite dependency is unknown. We found that SUP-35 directly binds to SUP-36, a homolog of SKP-1, a core component of the SCF ubiquitin ligase complex. This interaction is mediated by an F-box-like domain of SUP-35, which likely originated via convergent evolution. Interestingly, this interaction predated the evolution of toxicity, as we found that also RMD-2 directly binds to SUP-36. Remarkably, although SUP-36 interacts with the core SCF complex and canonical F-box proteins, disruption of SUP-36 binding to CUL-1 while preserving SUP-35 binding, did not abolish toxicity. Furthermore, by using cryo-EM and *in vitro* and binding assays, we showed that instead, SUP-36 enhances SUP-35 binding to SUP-37, thus acting as an allosteric activator. In agreement with this model, a close paralog of SUP-36 that is not required for toxicity, does not bind to SUP-35 *in vitro*.

In the absence of the antidote PHA-1, SUP-35 co-localizes with LEM-2, a marker for the nuclear lamina during embryogenesis. CoIP-MS experiments in these conditions revealed that SUP-35 interacts with its co-factor SUP-37 as well as with several other chromatin-binding proteins. Based on these findings, we speculate that SUP-35 kidnaps the essential DNA-binding protein SUP-37 and tethers it to the nuclear lamina to repress its cognate genomic loci. Taken together, our study illustrates how a novel tripartite molecular dependency arose from recycling and refinement of pre-existing protein interactions that are inherited via gene duplication.

427B Dissecting genetic components of heat stress-induced apoptosis and fertility levels using a new recombinant inbred line panel

Kristen A Quaglia[1], Emily Washeleski[2], Paul D Goetsch[2], Lisa N Petrella[11]Marquette University, [2]Michigan Technological University

As surface temperatures rise due to global climate change, organisms from rice to flies to nematodes face extinction solely due to the inherent temperature sensitivity of fertility. We are investigating how germline apoptosis may be a mechanism that can preserve fertility under temperature stress using genetically diverse wild strains of *C. elegans*. Under non-stress conditions, approximately 50% of oogenic nuclei in the germline undergo apoptosis to remove low-quality nuclei and/or to supply more cytoplasm to remaining oocytes in the N2 wildtype strain. It has been shown that as temperature increases and fertility decreases, apoptosis in the germline increases over baseline levels. We tested fertility and apoptosis levels in a variety of wild strains, and we found that fertility decreased, and apoptosis increased in all the strains under a moderate temperature stress, and there was a large range in both phenotypes. We also found a trend where strains with higher levels of fertility have higher levels of apoptosis at 26˚C. Since more apoptosis may be a method *C. elegans* use to maintain a higher level of fertility under temperature stress, we wanted to test if there is an underlying genetic component that influences the differences in fertility and apoptosis. We created a new recombinant inbred line (RIL) panel from two strains, ECA347 and NIC1107, that exhibit different levels of fertility and apoptosis under temperature stress. We evaluated fertility levels for each RIL strain at 26˚C and found a range in fertility in each of the lines. From this we found a significantly higher mean fertility in the strains with one of the mitochondrial backgrounds. We will be using this phenotype data to perform a genome wide association study to investigate single nucleotide polymorphisms (SNPs) associated with differences in fertility levels observed in our RILs. Our preliminary results indicate mitochondrial SNPs may be associated with differences observed in fertility levels when worms experience a moderate temperature stress. However, there also may be other genomic loci associated with better or worse fertility under temperature stress. Overall, this work will help to understand how organisms, like *C. elegans*, continue to reproduce despite experiencing global climate change.

428C Increased male production in *C. briggsae* hybrids without a *him* mutation

Karla Reyes Barajas, Joseph Ross Biology, California State University, Fresno

Correct chromosome segregation during meiosis is essential, because nondisjunction usually severely decreases the health of an organism. Paradoxically, nondisjunction of the X chromosome is essential to the generation of XO males in the *Caenorhabditis* genus. This has been interpreted to imply that nondisjunction can be adaptive and that species might evolve optimal nondisjunction rates, perhaps even just for a single nuclear chromosome: the X. To investigate the molecular basis of chromosome segregation regulation, we leveraged an anecdotal observation that hybridization of genetically diverse *C. briggsae* populations increases the frequency of spontaneous male production via virgin F1 hermaphrodites. In *C. briggsae* and other androdioecious nematode species like *C. elegans*, males are very rarely produced in natural populations, and their presence is a direct measure of the meiotic nondisjunction rate of the X chromosome. To formally investigate the observation that hybrid hermaphrodites throw more males, we crossed multiple genetically diverse temperate and tropical strains and measured the frequency of males produced by selfing virgin F1 hybrids. Our data show a significant increase in F2 male frequency from hybrid F1 hermaphrodites compared to F2 male frequency from control F1s, indicating that increased male frequency is not caused by a *him* mutation. A parental effect was also observed: one cross direction typically abolishes the increased male frequency. The frequency of embryonic lethality also increases in offspring of hybrid F1 hermaphrodites. These results suggest that genetic incompatibilities between diverse *C. briggsae* populations influence meiotic nondisjunction. Ongoing efforts seek to identify the identity of loci involved.

429C Conjugative plasmids are associated with commensal bacterial attachment to the intestinal epithelium

Dalaena E Rivera[1], Robert J Luallen[1], Marie-Anne Félix[21]Biology, San Diego State University, [2]Institut de Biologie de l'École Normale Supérieure

The persistence of conjugative plasmids in bacterial populations allows for bacteria to adapt to a wide array of environments, including host-associated niches. We discovered that a *C. elegans* commensal-like microbiome bacterium, *Lelliottia jeotgali* (LUAb3), contains a novel, conjugative plasmid that may be required for attachment to the intestinal epithelium. LUAb3 contains a plasmid (pLUAb3-52k) encoding for a type IV pilus (T4P) and a type 4 secretion system (for bacterial mating). T4P has been shown to allow intestinal attachment of certain pathogenic bacteria, but evidence for T4P in commensal attachment has not yet been shown. To determine the prevalence of this plasmid, we isolated three additional *Lelliottia* strains attaching to the intestines in other wild *Caenorhabditis* isolates from around the world and found that each of the *Lelliottia* isolates has a large plasmid divergent from pLUAb3-52k, but each contain the same highly conserved T4P operon and T4SS. These plasmids contain all the components for a retractable T4P, which is an ATP-powered appendage that extends and retracts to facilitate intimate host interaction. Interestingly, we found that other isolates of *L. jeotgali* and *Lelliottia* spp. do not contain plasmids and cannot colonize or attach to the intestine. Furthermore, BLAST analysis and phylogeny found there is higher conservation of *C. elegans*-associated plasmids to other host- or environmentally-associated bacteria than to each other. Altogether, the data suggests bacterial adherence to the *C. elegans* gut may be acquired through horizontal gene transfer via plasmids and that plasmids may allow for bacterial adherence in a variety of hosts. Findings from the research suggest that there may be a larger paradigm for animal microbiomes, whereby bacteria could gain the ability to become a part of the gut microbiome through plasmid acquisition.

430C Phoresis Survey of Nematoda Species In Soil Dwelling Invertebrates

Annika Samayoa, Woojoo Kim, Ethan Wang, Kristen Ngo, Nathaniel B Sutter La Sierra University

Despite small nematode species such as Caenorhabditis elegans and Oscheius tipulae are known to live and are commonly discovered in soil. Despite their small size, these nematodes exhibit remarkable adaptability and seek better habitats for survival and reproduction. This phoresis survey has been completed to investigate what species of soil nematode worms are being carried over on relatively larger soil invertebrates. Additionally, the investigation aimed to determine whether specific nematode species are associated with particular localized areas or specific soil invertebrate hosts. Soil invertebrates were collected from localized field sites and maintained under controlled laboratory conditions on lab-made Escherichia coli op.50 seeded Nematode Growth Media (NGM) for three weeks. Nematode presence was monitored, and isolated nematodes has been sequenced with 18S rRNA Primers. Preliminary result suggests that there is no correlation between particular soil invertebrates and nematode species.

431C More than gut feelings: neuroendocrine regulation of the gut microbiome

Ciara Hosea, Adrien Assie, Fan Zhang, Buck Samuel Baylor College of Medicine

The intricate relationship between gut health and host physiology represents a critical health determinant, with dysbiosis linked to numerous pathologies. Host-microbiome partnerships require sophisticated communication mechanisms, with the aryl hydrocarbon receptor (AHR) emerging as a pivotal mediator. Recent evidence reveals AHR's remarkable capacity to sense microbial metabolites directly within neuronal tissues, suggesting an elegant mechanism for neural monitoring of intestinal microbial dynamics.

We leveraged C. elegans to investigate this neural-intestinal axis, focusing on ahr-1, an AHR ortholog predominantly expressed in oxygen sensory neurons. When exposed to our custom-developed complex microbial community (BIGbiome), ahr-1 mutants exhibited distinctive gut microbiome alterations via 16S rRNA profiling. Transcriptomic analysis revealed the neuropeptide flp-8 as a key mediator of inter-tissue signaling downstream of ahr-1, with significant expression changes in URX oxygen sensory neurons in both genetic mutants and following adult-specific AID depletions of ahr-1.

Our findings demonstrate that reactive oxygen species (ROS) function as essential mediators between neuronal AHR activation and intestinal microbiome composition. Assessment of oxidative parameters revealed ahr-1 mutants exhibited elevated intestinal oxidative status when colonized with BIGbiome, despite minimal changes in stress response pathway activation. Individual bacterial strains demonstrated differential sensitivity to oxidative environments, suggesting a mechanistic explanation for selective microbiome restructuring through ROS-mediated selection pressures.

Our ongoing work aims to delineate the complete molecular signaling cascade through which neuronal ahr-1 senses microbial metabolites and orchestrates intestinal ROS levels to shape microbiome composition. These findings may inform therapeutic strategies for conditions associated with AHR dysregulation and intestinal dysbiosis, including inflammatory bowel disease and neurodegenerative disorders.

432C Comparing between synthetic and spontaneous tetraploid *C. elegans*.

Mara Schvarzstein[1,21]Biology, City University of New York, Brooklyn College and The Graduate Center, [2]Biology and Biochemistry, The Graduate Center at CUNY

Whole genome duplication (WGD) or polyploidization is a common developmental strategy for regulation of size, metabolism and cell function in specific cells and tissues (e.g. megakaryocytes in humans, hyp7 cell in *C. elegans*). Additionally, WGD is induced by stress in cells, tissues, and whole organisms, and it potentiates adaptation to stresses in normal and pathological contexts. Simplification of the process for generating viable tetraploid *C. elegans* strains from most diploid strains, offered the possibility of comparing isogenic strains that only differ in their ploidy. We have been exploring the potential of utilizing these synthetic tetraploid strains as tractable model for Organismal polyploidization and its effects. We will share our progress on the characterization of the tetraploid animals generated in the laboratory. Additionally, we will discuss the differential fitness between the synthetic tetraploids and a strain that spontaneously became tetraploid under both in normal culture conditions and under stress conditions. This analysis is aimed at assessing whether, and under what conditions, synthetic tetraploid nematodes are adequate models for organismal WGD in nature.

433C Independent mechanisms of benzimidazole resistance across *Caenorhabditis* nematodes

Amanda Shaver[1], Ryan McKeown[2], Joyce M Reyes Otero[3], Erik C Andersen[11]Johns Hopkins University, [2]Northwestern University, [3]University of Puerto Rico

Benzimidazoles (BZs), a widely used class of anthelmintic drugs, target beta-tubulin proteins, disrupt microtubule formation, and cause nematode death. In parasitic nematode species, mutations in beta-tubulin genes (*e.g.*, *isotype-1 beta-tubulin*) are predicted to inhibit BZ binding and are associated with BZ resistance. Similarly, in the free-living nematode *Caenorhabditis elegans*, mutations in an *isotype-1 beta-tubulin* ortholog, *ben-1*, are the primary drivers of BZ resistance. The recurrent association of BZ resistance with mutations in beta-tubulin genes is an example of repeated evolution of drug resistance across diverse nematode species. To evaluate this hypothesis, we identified predicted BZ resistance alleles in beta-tubulin genes across wild strains from three *Caenorhabditis* species: *C. elegans*, *C. briggsae*, and *C. tropicalis*. We hypothesized that if these species experienced similar selective pressures, they would evolve resistance to BZs by mutations in any of three beta-tubulin genes (*ben-1*, *tbb-1*, and *tbb-2*), which are expressed in multiple tissues and implicated in tubulin polymerization dynamics. Using high-throughput development assays, we tested the association of natural beta-tubulin alleles with BZ resistance. We found that a heterogeneous set of variants identified in *Cel-ben-1* were highly associated with BZ resistance. In *C. briggsae*, only two variants unique to Cbr-ben-1 (W21stop and Q134H) were associated with BZ resistance. In *C. tropicalis*, two unique missense variants were identified in *ben-1*, but neither was associated with BZ resistance. Because no variants in *ben-1* were associated with BZ resistance in *C. tropicalis*, we tested whether predicted mutations in *tbb-1* and *tbb-2* were associated with BZ resistance in the three species. We found that *C. elegans* has no variants in *tbb-1* or *tbb-2*, and that variants in *tbb-1* or *tbb-2* in *C. briggsae* and *C. tropicalis* were not associated with BZ resistance. Our findings reveal a lack of repeated evolution of BZ resistance across the three *Caenorhabditis* species and highlight the importance of defining BZ resistance mechanisms outside the beta-tubulin gene family. These results also suggest that BZ selection in the niches of these three species are likely distinct.

434C Ecological, molecular and comparative insights into *Caenorhabditis brenneri* as a complementary model organism to *C. elegans*

Md. HASANUZZAMAN Talukder[1], Md. Rajiur Rahaman Rabbi[2], Md Mahfuzur Rahman Sajib[2], Md Makshuder Rahman Zim[2], Mostak Ahmed[2], Nurnabi Ahmed[2], Babul Chandra Roy[2], Muntasir Kamal[31]Department of Parasitologyy, Bangladesh Agricultural University, [2]Bangladesh Agricultural University, [3]Krembil Research Institute

The *Caenorhabditis elegans* is used as a model in the advancement of cell and molecular biology, drug discovery and host-microbe interactions. However, its sibling *C. brenneri*, remains relatively underexplored despite its hyperdiverse genome and ecological significance. This study aimed to investigate *C. brenneri* through ecological surveys, genetic characterization and biological comparisons with *C. elegans (N2)* to position it as a complementary model organism. About 11 distinct bio-ecological zones were selected for sampling in cooler habitats, samples were shipped to the laboratory, live nematodes transferred to OP50 *E. coli* seeded NGM plates, isolated and allowed to proliferate at 20°C. A comparative analysis of both species including lifespan, fecundity, thermo-tolerance, anthelmintic sensitivity and bacterial fast-killing assay (FKA) was conducted to evaluate *C. brenneri* as a complementary model to *C. elegans*. Distinct morphological traits in both sexes help in primary identification and worm's DNA succeeded to amplify ITS2 and 18s rRNA regions confirmed *C. brenneri*. The comparative study revealed that *C. brenneri* live longer and produce fewer eggs than *C. elegans.* Additionally, *C. brenneri* exhibited higher survival under prolonged heat stress, significantly greater survival against *P. aeruginosa* and *S. aureus* infections and slower mortality rates in anthelmintics treatment compared to *C. elegans*. This pioneering investigation confirms the availability of *C. brenneri* in different geography of Bangladesh, outlines its evolutionary context and resilience to stressors. These findings highlight its potential as a robust complementary model for research into species-specific responses to environmental and pathogenic stress, lead-compound discovery, and comparative genomics.

435C Moving into the pangenomics era: population genomics of the hyperpolymorphic *Caenorhabditis brenneri* using variation graphs

Anastasia Teterina[1], John H Willis[2], Angel G Rivera-Colón[1], Peter L Ralph[1,3], Andrew D Kern[1], Patrick C Phillips[11]Institute of Ecology and Evolution, University of Oregon, [2]Institute of ecology and Evolution, University of Oregon, [3]Department of Data Science, University of Oregon

Caenorhabditis brenneri, an outcrossing nematode in the Elegans subgroup (Rhabditida; Sudhaus & Kiontke 2007), is one of the most genetically diverse eukaryotes, with nearly one in ten nucleotides being polymorphic—comparable to bacterial population diversity levels (Dey et al. 2013). Patterns of genomic variation provide key insights into species' evolutionary histories, but decoding these signals is challenging, especially in hyperpolymorphic species. High levels of genetic and structural variation complicate the population genetic analysis, as most standard approaches focus on diallelic single-nucleotide variants and exclude other forms of variation. We address these challenges by generating high-quality individual genome assemblies from long reads and using pangenomic approaches to better capture population diversity in *C. brenneri*. Variation graphs, which represent genomic variation as a network of interconnected sequences rather than changes in a single linear reference, allow us to integrate diverse haplotypes, improving the resolution of structural variation, complex variants, multiple alleles, and gene evolution. We examine several aspects of *C. brenneri* population genetics: (1) genome-wide diversity and its distribution within genetic elements, using variation graphs to incorporate indels and complex variants into population analyses; (2) structural variation patterns along the genome and their impact on gene orientation, order, and copy number across strains; (3) exon-intron variation in single-copy genes within the Elegans group to explore gene structure evolution at the population level; and (4) diversity and divergence within and across geographic locations to explore population structure. Our findings provide new insights into *C. brenneri*'s population history and deepen our understanding of genome and gene organization evolution in nematodes.

436C The developmental plasticity switch locus *eud-1* is a mutational hotspot in natural isolates of the nematode *Pristionchus pacificus*

Penghieng Theam, Hanh Witte, Christian Rödelsperger, Ralf Sommer Integrative Evolutionary Biology, Max Planck Institute for Biology Tübingen

Developmental plasticity is widespread in nature and its evolutionary significance is studied across all domains of life. Underlying molecular mechanisms are increasingly characterized with several case studies that also provide support for the recurrent evolution of plasticity. For example, the facultative predatory nematode *Pristionchus pacificus* exhibits mouth-form plasticity controlled by the developmental switch gene *eud-1*. Investigations of *P. pacificus* natural isolates revealed that divergent combinations of *cis*-regulatory elements at the *eud-1* locus are responsible for the expression of alternative phenotypes in spatially and historically separated populations. However, the temporal scale of population differentiation and the genetic target space for *P. pacificus* plasticity evolution remain currently unknown. Here, we investigate natural isolates from a new habitat on La Réunion Island over a 10-year period and find novel, independent variants at the *eud-1* locus. Through CRISPR-engineering we show that an exonic 19 bp deletion affects the signal peptide of EUD-1 and is accompanied by alternative transcriptional start sites. Further genome sequencing of natural isolates from that locality identified a variant with a deletion in *eud-1* resulting in a gene knockout and a predation-defective strain. This work suggests that the *eud-1* switch locus is a mutational hotspot for *P. pacificus* mouth-form plasticity.

437C Exploring the mechanisms through which MSS glycoproteins modulate sperm competitiveness

Asan Turdiev, Justin Van Goor, Savannah Speir, Jillian Manning, Eric Haag Biology, University of Maryland, College Park

Sperm competition is found across multicellular organisms using external and internal fertilization. Research from the past decades on several animal models, including flies, mice, and nematodes, has led to the identification of multiple genes with apparent roles in sperm competitiveness. Their molecular mechanisms are generally unknown but appear distinct from core fertilization factors and may reveal general lessons about gamete biology. *Caenorhabditis* nematodes are ideal organisms for functional characterization of these genes. Previously, we showed that male secreted short (MSS) glycoproteins enhance the competitiveness of male sperm. In obligately outcrossing *Caenorhabditis*, MSS is dispensable for baseline fertility but required for intraspecific sperm competitiveness. MSS is lost in self-fertile lineages, likely as a response to selection for a hermaphrodite-biased sex ratio. However, genetic restoration via transgene in the self-fertile nematode *C. briggsae* is sufficient to make MSS+ male sperm more competitive. Recently we showed that other mss-related proteins (MSRPs) found in the *C. briggsae* genome are similar in structure, expression, and localization to MSS, but are not necessary for normal sperm competitiveness. A closer examination of MSS and MSRP from multiple species identified small but evolutionary conserved differences in the signal peptide sequence. Given that MSS but not MSRPs provide enhanced sperm competitiveness upon expression we hypothesize that the difference in apparent functions is the consequence of different glycans added to both proteins post-translationally. Using molecular, biochemical, and fluorescent imaging tools on newly generated single-copy transgenic *C. elegans* we attempt to interrogate: 1) what is the contribution of predicted glycosylation sites on MSS-dependent sperm enhancement; and 2) how does the sequence of signal peptides influence glycosylation in the endoplasmic reticulum of sperm cells. This work not only has the potential to elucidate the functional contribution of specific sugar moieties on protein function but also to expand our understanding of protein biogenesis in cells in general.

438C Hitchhiking Bacteriophages use Nematodes to Travel Through Spatially Structured Habitats

Lisa van Sluijs[1], Cassidy Dietz[1,2,3], Floris van Noort[2], Johannes Helder[1], Mark P. Zwart[4], Kyle Mason-Jones[21]Laboratory of Nematology, Wageningen University and Research, Wageningen University and Research, [2]Department of Terrestrial Ecology, Netherlands Institute of Ecology, [3]Department of Natural Resources and the Environment, University of New Hampshire, [4]Department of Microbial Ecology, Netherlands Institute of Ecology

Bacteriophages (phages) infect bacteria and are likely a major driver of microbial community compositions in soils. However, to infect new bacterial hosts, phages need to travel through a hostile environment, overcoming a diverse range of spatial and physical barriers without the benefit of active motility or sensory perception. Pioneering evidence in laboratory settings showed that phages may hitchhike on motile organisms, including nematodes. Here, we tested whether nematodes also vector phages through the soil labyrinth. First, we demonstrated that *Caenorhabditis elegans* transferred the laboratory model phage T7 and the soil phage Φ ppu-W11 on agar. Second, we confirmed that two mechanisms contribute to phage transfer: intestinal intake and cuticular attachment. Presence of host bacteria was not necessary for phage vectoring by nematodes. Third, phage vectoring by *Caenorhabditis* nematodes was tested in microcosms containing compost and sandy soil. In these spatially and physically complex habitats, nematodes were critical for moving phages. Together, these experiments demonstrate a previously unknown mechanism for interactions between nematodes and phages within the soil microbiome.

439C Initial sampling of terrestrial nematodes in Hong Kong and Southern China to assess their biodiversity and potential as an indicator for soil health

Zhongying Zhao[1], Tongshu Wen[2], Yiqing Liu[11]Hong Kong Baptist University, [2]BIOL, Hong Kong Baptist University

The phylum Nematoda is one of the most abundant and diverse metazoan taxa on Earth. Advances in sequencing techniques allow cost-effective classification and DNA barcoding of wild-isolated nematodes, which greatly facilitates the investigation of the biodiversity and evolutionary history of nematode species. In this study, we collected nematode soil samples from over 80 different locations, mainly from Hong Kong and Southern China. We identified a total of 30 nematode species, which covers species in Rhabditomorpha and Diplogasteromorpha in Clade V, Cephalobomorpha, Panagrolaimomorpha, and Tylenchomorpha in Clade IV, and Monhysterida in Clade C. The taxonomic classification is based on the sequencing of ribosomal DNAs, especially the internal transcribed spacer (ITS) sequences, by the Sanger DNA sequencing or Oxford Nanopore Technologies. Although most of the identified nematodes are apparently free-living, Alloionematidae and Aphelenchoididae species appear parasitic, as judged by the inference from their closely related species. Eight of them are likely to be new species because they show only modest similarity in their rDNA sequences to those in the existing databases. Partial cultured strains have been morphologically characterized by either differential interference contrast (DIC) microscopy, scanning electron microscope (SEM), or their combination. The isolated species and strains study provided a glimpse into the nematode biodiversity in these regions, reflecting the soil health status in the areas sampled. The results can be integrated with other indexes for ecological monitoring. The successively cultivated species and strains constitute invaluable resources for the study of nematode phylogenomics and population genetics in the community.

440C Variation in the genomic architecture of Oxidative Stress Resistance between *C.elegans* strains

John Willis, Anastasia Teterina, Megan Moerdyk-Schauwecker, Christine Sedore, Anna Coleman-Hulbert, Erik Johnson, Kristin Robinson, Patrick Phillips Institute of Ecology and Evolution, University of Oregon

The genomic architecture of phenotypic variation among populations is dependent on heritable mutations that determine the individual's ability to respond to environmental conditions. Using oxidative stress as an environmental perturbation, we discovered that the N2 and MY16 strains differ in their ability to survive exposure to paraquat and hydrogen peroxide (H2O2) in a strain-specific manner. MY16 is more resistant to superoxide production from paraquat (PQ) exposure then N2 while N2 is more resistant to H2O2 exposure then MY16. This result was surprising since superoxides are known to be quickly converted to H2O2 and less toxic oxygen species by enzymatic means. To identify casual alleles that confer paraquat resistance to MY16 we constructed F12 recombinant pools between parental strains for a modified bulk segregant analysis approach we call Evolutionary Segregant Analysis (ESA). Like others, we used obligate outcrossing lines (*fog-2*(-)) of each strain for construction of the pools in addition to doing multiple rounds (n=5) of selection with controls. Mapping data conferred a single peak on LGV containing the aldehyde oxidase, *gad-3*, which differs in two mis-sense mutations between the parental lines. CRISPR based engineering of the endogenous *gad-3* gene in each parental background confirmed the causative nature of the amino acid differences to PQ resistance: MY16 GAD-3 makes N2 more resistant to PQ while N2 GAD-3 makes MY16 more sensitive to PQ. Furthermore, the MY16 GAD-3 version makes N2 more resistant to H2O2 suggesting that this allele of *gad-3* is not responsible for N2›s greater resistance to H2O2. To identify the causative alleles the confer N2 more resistant to H2O2. we performed ESA analysis with the same pools but for survival to H2O2 exposure and are currently in the process of finalizing these mapping results.

441C Potential amplification of collagen gene copy number in the elongated *Caenorhabditis inopinata*

Gavin Woodruff, Biological Sciences, University of Oklahoma

Understanding the genomic drivers of morphological change is a central goal of evolutionary developmental biology. *Caenorhabditis inopinata* is an exceptional member of its genus, with adults exhibiting a dramatic increase in length compared to its close relatives. Two previous transcriptomic studies reported extensive divergence in collagen gene expression among *C. inopinata* and *C. elegans*. Additionally, some collagen genes regulate body size and shape in *C. elegans*, and a body size TGF-β signaling pathway also regulates the activity of many cuticle collagens in *C. elegans*. Taken together, this suggests that collagen genes may be important evolutionary substrates of body shape divergence. To address this possibility, I estimated the number of collagen genes among the publicly-available genomes of 54 *Caenorhabditis* species. *C. inopinata* had 184 collagen genes, greater than two standard deviations from the mean (145; among *Caenorhabditis* species with <30,000 genes). Inference of orthologous groups of collagen genes, in tandem with an examination of the genomic location of collagen genes in *C. inopinata*, revealed potential lineage-specific duplication events. Patterns of synteny suggest the existence of a highly-conserved and ancient cassette of three collagen genes that are homologous to *col-144*, *col-145*, and *col-10* in *C. elegans*. This three-gene cassette is largely stable across phylogeny, found in eleven *Caenorhabditis* species with high quality assemblies (including members of the Japonica group, Elegans group, as well as *C. bovis*; although this cassette has been reduced to one collagen gene copy in *C. niphades*). In *C. inopinata*, this three-gene cassette has expanded to ~13 copies, likely enabled via transposable element activity. Additionally, at least one additional collagen gene cassette (*col-167*, *col-168*, *col-169*, *col-170*, and *col-171*) has expanded from five copies in *C. elegans* to ten copies in *C. inopinata*. RNA-seq data reveal these homologous genes to have divergent transcriptional dynamics between *C. elegans* and *C. inopinata*. To understand the role of these genes in body size regulation, I generated a 5,941 bp deletion removing the entire *col-144/145/10* cassette in *C. elegans*. While there are as yet no obvious impacts on body size, preliminary observations suggest this mutation may promote embryonic inviability and slow growth. Ongoing reverse genetic work that directly manipulates collagen copy number in *C. elegans* and *C. inopinata* will also be discussed. Regardless, this work suggests the possible existence of a lineage-specific amplification of a highly-conserved and ancient collagen gene cassette in a species with a novel body shape. Changes in collagen gene copy number may be connected to the evolution of large body sizes in this group.

442C Investigating the evolution of transcriptional regulation underlying morphogenesis

Raya Jallad[1], Yash Patel[1], Ritsuka Ting[1], Karin Kiontke[1], David H.A. Fitch[1], Alyssa Woronik[21]Biology, New York University, [2]Sacred Heart University

Morphogenesis, or the development of form, is a universal process during development of multicellular organisms and is regulated by the precise spatiotemporal expression of genes within gene regulatory networks (GRNs). Because GRNs control cell behaviors at the transcriptional level, the key points involved in GRN evolution are proposed to be transcription factors, their regulation, and their interaction with target genes. While studies in model organisms have significantly improved our understanding of how GRNs regulate development, a major remaining knowledge gap is understanding how GRNs evolve. One theory for GRN evolution, known as the hotspot hypothesis, predicts that GRN architectures bias GRN evolution, such that the same genes will be repeatedly co-opted during convergent evolutionary events . Our work aims to test the hotspot hypothesis using a male-specific morphogenetic process, known as Tail Tip Morphogenesis (TTM), which evolved independently in the lineage to *C. elegans* and multiple other nematode lineages. During TTM, which occurs during the L4 stage, the hypodermal tail tip cells in males change shape and then migrate internally. TTM does not occur in females/hermaphrodites, which retain the pointed shape of the larval tail tip. In *C. elegans* a DM-domain transcription factor, DMD-3, is required and sufficient for TTM and is therefore the key regulator. Here we test the prediction that DMD-3 has been co-opted as the key regulator during a presumed independent evolution of TTM in the lineage to *Oscheius tipulae* by knocking out the DMD-3 ortholog in this species via CRISPR/Cas9 gene editing. Additionally, to investigate the TTM GRN and its evolution, we developed a novel, single-tissue RNA-seq method to sequence the transcriptome of individual tail tips over a developmental time course. Pioneering this method in *C. elegans*, we were able to construct profiles of mRNA abundance over time for genes expressed in the tail tip during TTM. This analysis provides insights into the genes within the TTM GRN and its architecture. We are currently applying this methodology to *O. tipulae*. Our goal is to compare the transcriptional regulation and architecture of the TTM GRN between these species to gain insights into GRN evolution.

443C Pan-genome analysis reveals protein-degradation pathways as key drivers for inter- and intraspecific divergence

Jeffrey, Dongying Xie, Pohao Ye, Yiming Ma, Zhongying Zhao Hong Kong Baptist University

Genomes continuously evolve in response to diverse internal and external challenges. Despite constant genomic variation, species maintain their specific genetic identity, whereas hybridization typically compromise such integrity, resulting in phenotypical inferiority such as hybrid incompatibility (HI). The evolutionary forces and molecular mechanisms that drive genomic adaptations while simultaneously induce genomic conflict among species or populations remain largely enigmatic. Although we previously leveraged the nematode species *Caenorhabditis briggsae* and *C. nigoni* as model systems to study HI and provided tremendous implications underlying their speciation, the genomic foundation of their sequence divergence and consequent reproductive barriers is still poorly understood. In this study, we assembled high-quality genomes for the recently collected *C. nigoni* wild isolates and conducted a pan-genome-level comparative analysis across populations of both species. We observed that *C. nigoni* strains consistently harbor larger genomes and more genes than those of *C. briggsae*, a pattern that aligns with their asymmetrical gene flow. The increased genome sizes of *C. nigoni* strains is primarily attributed to disproportionately large unaligned regions, whereas the higher gene numbers stem from an extensive expansion of *C. nigoni*-specific dispensable genes. Interestingly, both features are largely driven by a *C. nigoni*-specific expansion of protein degradation-related genes, particularly F-box genes, which likely reflect distinct selective pressure and differential immune responses between the two nematodes. We finally characterized in detail of an F-box gene family, i.e., the *fbxn* gene family which contains the very first interspecific HI gene we recently identified between the two species, and demonstrated how the extensive polymorphism within this gene family contributes to the establishment of reproductive barriers. Collectively, our findings illuminate the genomic basis of divergence between the two nematode species, emphasizing the pivotal roles of protein degradation and immune responses in speciation.

444C Meiotic chromosome pairing and parent-of-origin allele silencing in *C. briggsae* and *C. nigoni* interspecies hybrids

Charlotte Choi, Rayka Yokoo, Neuza Matias, Sophia Mirda, Anne Villeneuve Stanford University

We have been developing *C. briggsae* / *C. nigoni* interspecies hybrids as a system for investigating 1) the evolution and modularity of the meiotic machinery and 2) mechanisms underlying homologous chromosome recognition and pairing. To this end, we used a computational strategy to identify pairing center motif sequences in both species, including motifs on the *X* chromosomes where the pairing center binding protein HIM-8 may bind. Further, we established CRISPR editing in *C. nigoni* and used pre-existing methods in *C. briggsae* to create strains expressing tagged versions of key meiosis proteins, including HIM-8, from their endogenous loci. Using these strains, we found that *C. nigoni* HIM-8 can functionally substitute for *C. briggsae* HIM-8 in mediating associations between the *Cni-X* and *Cbr-X* chromosomes in F1 hybrids. Interestingly, there appears to be a non-linear increase in accumulation of HIM-8 that occurs upon contact between the homologs.

In the course of this work, we found that expression of some tagged germline-specific proteins in the *C. briggsae*/*C. nigoni* F1 hybrids appears to depend on the parental origin of the allele. Our working hypothesis is that paternally-derived alleles of some genes may be silenced in F1 hybrids by a mechanism that depends on the piRNA repertoire present in the germ line of the maternal parent. We are currently testing various predictions of this model by comparing the small RNA and mRNA repertoires of the parental species and of F1 hybrids derived from reciprocal crosses. Together these results may begin to provide insights into the mechanisms by which reproductive incompatibility arises.

445C Experimental evolution in multispecies communities

Peter C. Zee, Ryan P. Phillips Department of Biology, University of Mississippi

Species interactions are a ubiquitous feature of ecological communities. As such, understanding the outcomes of these interactions provides important information for the maintenance of biodiversity. Since the outcomes of the interactions are mediated by organismal traits, understanding how these traits evolve over time will be key to understanding biodiversity dynamics over time. Here, we are particularly interested in understanding whether responses to selection in multispecies communities is qualitatively different than expectations from individual pairwise interactions. To better understand the response to selection in ecological community context, we evolved *C. elegans* populations in different bacterial prey environments. We experimentally evolved 20 populations of *C. elegans* across 3 distinct prey treatments (=60 total evolved populations). We used the genetically diverse *C. elegans* strain A6140 (dervied from a mixture of 16 natural isolate founders) as our ancestor, and *C. elegans* Microbiome Resource (CeMBio) bacterial strains JUb44 and CEent1 (as prey either in isolation or a 1:1 ratio) as our selection regimes. We will present results both on phenotypic responses to selection in worms (population growth rates and feeding rates) and compare whole-genome resequencing data of our derived populations across treatments with the ancestor populations to understand if genomic regions under selection differ among selection regimes. Results from this work will give insight into the nature of responses to selection in an ecological community context as well as information about selectional pleiotropy across prey different environments.

446A Transcriptome and translatome profiling of domain-specific *lotr-1* alleles to better understand the balance of epigenetic signals in the germline.

Kehinde Abayomi, Noah Lind, Catherine Sharp, Dustin Updike The Mount Desert Island Biological Laboratory

The LOTUS and Tudor domain-containing protein LOTR-1 is most similar to mammalian TDRD5, a protein whose mutation leads to male-specific sterility. In *C. elegans*, LOTR-1 is a component of Z granules and works with ZNFX-1 to distribute small RNAs across WAGO and mutator targets. Z granule activities are implicated in transgenerational epigenetic inheritance (TEI). To better understand gene and protein expression changes in full and domain-specific *lotr-1* mutants, we performed mRNA sequencing on total lysate and polysome fractions from wild-type and mutant young adult hermaphrodites. Our analysis revealed both domain-specific and shared changes in gene expression, as well as alterations in the translational efficiency of known TEI targets. We are further investigating these expression changes using smFISH and fluorescent reporters on endogenous targets within the context of development.

447A The G3BP1 ortholog regulates the degradation of select circular RNAs in *C. elegans*

Emmanuel Adeyemi, Hussam Alshareef, Alexander M van der Linden Biology, University of Nevada, Reno

Circular RNAs (CircRNAs) are covalently closed RNA molecules produced from a separate form of alternative splicing known as back-splicing and are a prime example of RNA transcripts that accumulate during organismal aging. CircRNAs are resistant to degradation by exonucleases due to the absence of 5' or 3' ends. Furthermore, the age-dependent accumulation of circRNAs has been linked with various diseases, including cancers and multiple neurodegenerative diseases. Despite reported circRNA degradation processes, the precise mechanisms underlying circRNA degradation remain poorly understood. The stress granule (SG) assembly factor and RNA-binding protein, G3BP1, as well as the endonuclease decay factor of the Nonsense Mediated mRNA Decay (NMD) pathway, SMG-6, have been associated with circRNA degradation. SGs are ribonucleoprotein complexes that assemble to protect an organism's transcriptome and proteome in response to extracellular stress. An ortholog of G3BP1 exists in the *C. elegans* genome called *gtbp-1*, which contributes to SG formation in worms. Our findings suggest that mutations in *gtbp-1* reduce the expression of a select group of circRNAs that accumulate during aging in *C. elegans,* including circ-*crh-1*, which is involved in mean lifespan regulation (Knupp *et al.* 2022). Moreover, we discovered that loss of *gtbp-1* suppresses the accumulation of select circRNAs in animals lacking the NMD endonuclease decay factor, *smg-6*. In addition to higher levels of circRNA accumulation in the *smg-6* animals compared to the SG mutants, we also found increased localization of a fluorescent SG reporter protein, suggesting a potential link between SGs and increased circRNA accumulation. Lastly, we conducted RNA-pulldown to identify RNA-binding proteins bound to the GTBP-1-regulated circRNA, circ-*crh-1*, using biotin-tagged antisense oligonucleotides. Our findings could provide insight into the mechanisms underlying circRNA degradation.

448A Perinuclear germ granules as regulators of RNAi-targeted transcript fate

Saima Akhter[1], Alicia K Rogers[21]BIOLOGY, University of Texas at Arlington, [2]Biology, University of Texas at Arlington

RNA interference (RNAi) pathways regulate gene expression by silencing transposable elements and ensuring proper germline gene expression. Perinuclear germ granules act as hubs for RNA-induced silencing complex (RISC)-mediated mRNA processing, including small RNA amplification for target silencing. In *C. elegans*, distinct branches of the RNAi pathway either repress or promote gene expression, and proper sorting of RNAs between these pathways is essential for fertility. However, the molecular mechanisms governing RNA sorting and pathway selection remain poorly understood. In *D. melanogaster*, it has been shown that a transcript's proximity to the piRNA machinery in nuage is sufficient to trigger piRNA production, suggesting that transcript processing may be influenced by subcellular localization within germ granules. This raises the possibility that RNAi-mediated transcript fate is not solely dictated by small RNAs but also by the spatial organization of RNAi factors. We hypothesize that RNA sorting into RNAi branches is mediated by transcript shuttling between perinuclear germ granules. To test this hypothesis, we have adapted an *in vivo* heterologous RNA-to-protein tethering assay for germline-specific expression in *C. elegans*. This system allows us to tether a reporter transcript to the core components of the germ granules, enabling targeted recruitment of transcripts to germ granules and systematic assessment of the impact on transcript processing. Our preliminary results reveal that recruitment of a reporter transcript to distinct germ granules elicits different regulatory outcomes, suggesting that perinuclear granules are not passive sites of RNA processing but actively contribute to transcript fate determination. To further characterize these effects, we are performing quantitative RT-PCR to measure reporter transcript levels and small RNA sequencing to assess the initiation of small RNA production. If recruitment triggers reporter transcript silencing, we will use chromatin profiling to assess changes in the epigenetic landscape at the reporter's genomic locus. These experiments provide a framework for understanding how spatial compartmentalization within germ granules influences RNAi-mediated gene regulation. Ultimately, understanding these mechanisms will shed light on the broader principles of RNA-based gene regulation and may provide insights into fertility regulation and RNA dysregulation in disease contexts.

449A Investigating the Small RNA Loading Preferences of *C. elegans* Argonaute Proteins.

Khlifa Alnaim, Carolyn Phillips University of Southern California

RNA interference (RNAi) is a gene regulation pathway essential for proper development, cell differentiation, and cell function. The effector protein, known as an Argonaute, binds to a small RNA and forms a complex to silence transcripts complementary to that bound small RNA. In *C. elegans*, there are 19 functional Argonaute proteins with overlapping and non-overlapping expression patterns that associate with distinct populations of small RNAs. However, the mechanisms driving the association between Argonaute proteins and specific small RNA populations remain unclear. The Argonaute protein CSR-1 has two isoforms: CSR-1A, which is expressed in L4s, and CSR-1B, which is expressed in L4s and adults. These isoforms associate with small RNAs that are biochemically identical but target distinct genes. To investigate whether the differential small RNA association is due to different temporal expression patterns of CSR-1 isoforms, we ectopically expressed CSR-1A throughout the germline to investigate whether this alters the canonical small RNA populations bound by CSR-1A. CSR-1B is an essential protein required for fertility, as loss-of-function mutations in CSR-1B render animals completely sterile. Preliminary results show that the ectopic expression of CSR-1A throughout the germline rescues the sterility phenotype found in CSR-1B mutant animals. Another example of the differential small RNA association between Argonaute proteins is the paralogs WAGO-1 and WAGO-4. They share expression profiles but differ in their small RNA association. We aim to use structural domain predictions to create WAGO-1 and WAGO-4 protein chimeras to better understand how sequence differences between Argonaute proteins influence small RNA binding preferences. All in all, this project aims to elucidate the mechanisms behind germline Argonaute binding specificity in *C. elegans* through a molecular and structural lens, which will elucidate the relationship between Argonaute dysfunction and human diseases. Lastly, understanding the connection between sequence, structure, and function in Argonaute proteins can support the current gene therapy efforts that utilize engineered or modified Argonaute proteins.

450A The Phosphoregulation of an H4K20-specific Demethylase in Dosage Compensation of *C. elegans*

Anati Alyaa Azhar[1], Zoey Tan[2], Gyorgyi Csankovszki[11]University of Michigan, Ann Arbor, [2]MCDB, University of Michigan, Ann Arbor

In species that use chromosome-based sex determination, there is a difference in the number of sex chromosomes that lead to an unequal amount of gene expression between sexes. To avoid sex-specific lethality, it is essential to equalize this gene dosage difference through a process called dosage compensation. In *C. elegans*, hermaphrodites (XX) downregulate both their X chromosomes by half to equalize them to the single X chromosome in males (XO). This dosage compensation process is primarily conducted by the Dosage Compensation Complex (DCC), a multimeric complex of proteins consisting of a Condensin IDC and five other accessory proteins that bind to the hermaphrodite X chromosomes.

One of the non-condensin members of the DCC, DPY-21, is an H4K20-specific demethylase that binds to dosage compensated X chromosomes and its activity leads to X-enrichment of H4K20me1. Although DPY-21 plays an important role in the compaction and downregulation of the hermaphrodite X chromosomes, less is known about its regulation. While Condensin IDC binds to the X chromosomes at the 30-cell stage of embryonic development, DPY-21 localizes around the bean stage. Additionally, while Condensin IDC localizes to the X chromosomes throughout the cell cycle, DPY-21 only localizes to the X in interphase. Previous research identified three putative phosphosites on DPY-21 : Thr565, Ser775/Ser776, Thr1329 with the latter most located near the JmjC demethylase domain. In order to determine if these putative phosphosites play a role in the localization and demethylase function of DPY-21, we used CRISPR/Cas9 site-specific mutagenesis to mutate these residues into alanine to render them non-phosphorylatable. We aim to characterize how these phosphodead mutants of DPY-21 affect DPY-21's localization and enrichment of H4K20me1 on dosage compensated X chromosomes in hermaphrodites.

451A Importin β mRNA and its encoded protein both localize to the nuclear periphery during early *C. elegans* embryogenesis

Ambika Basu, Lindsay P. Winkenbach, Nora Tayefeh, Erin Osborne Nishimura Biochemistry and Molecular Biology, Colorado State University

Local translation is an understudied aspect of gene expression, cell biology, and development. During *Caenorhabditis elegans* embryogenesis, the maternally inherited mRNA *imb*-2 (Importin beta-2) concentrates around the nuclear periphery together with its encoded nuclear import protein. This sub-cellular localization pattern raises two questions: How is *imb-2* mRNA directed to the nuclear periphery, and does its accumulation there arise for some function? To learn which signals drive *imb-2's* localization, we used single-molecule Fluorescence In Situ hybridization (smFISH) combined with molecular biology and genetics approaches. We found *imb-2's* 3'UTR was not sufficient to drive localization of an appended naïve reporter, suggesting 3'UTR mRNA sequences were dispensable to direct localization. To assess whether translation of the IMB-2 protein is required to localize the *imb-2* mRNA, we assessed *imb-2* mRNA localization after treatment with different translation inhibiting drugs. These experiments showed that disrupting translation led to loss of the *imb-2* mRNA localization unless the nascent chain complex was stabilized. This suggests that an intact ribosome-nascent chain complex (the nascent peptide chain, the ribosome, and the associated *imb-2* mRNA) has some sequence information that is guiding the mRNA and the protein to the nuclear periphery. To further explore if the mRNA or amino acid sequences were necessary for mRNA localization, we re-coded the *imb-2* mRNA coding sequence while retaining a synonymous, wild-type IMB-2 amino acid sequence by virtue of redundancy in the genetic code. We observed that our re-coded *imb-2* exhibited variable mRNA instability and localization. Taken together, this suggests *imb-2* depends on a combination of mRNA- and amino acid-encoded information to ensure both its mRNA stability and localization at the nuclear periphery. Current experiments are aimed at identifying the mRNA sequences critical for mRNA stability, the discreet IMB-2 peptide motifs responsible for localization, and the impact of ectopically translating *imb-2* at other locales.

452A Unraveling the roles of tRNA-derived fragments in animals

Jade Beaudoin-Fredette, Pauline Adjibade, Vaishnav Vasudevan, Martin Simard CHU de Québec-Université Laval Research Center

While research on short non-coding RNAs has significantly advanced our understanding of molecules like interfering RNAs and microRNAs, our knowledge of another class of small RNAs derived from transfer RNAs remains limited. tRNA-derived fragments (tsRNAs) have been shown to accumulate in cells, with their levels modulated in a context-dependent manner. Recent studies suggest that these molecules are involved in regulating various molecular processes particularly in stress conditions. To better understand the biological function of these fragments, it is crucial to comprehend their production mechanisms and identify key partners involved in their function. To achieve this, we use the *Caenorhabditis elegans* model, which is known to contain an abundance of tsRNAs. Through high-throughput small RNAs sequencing analysis of young adult *C. elegans*, we identified five subclasses of tsRNAs, with 5′ tsRNA halves, originating from the 5′ end of mature tRNAs and cleaved at the anticodon loop, being the most abundant.

The first objective of this project is to determine how these fragments are produced. To achieve this, we will investigate the role of 38 *C. elegans* ribonucleases in the maturation of the three most prevalent 5› halves tsRNAs using RNAi. We have established that these three fragments can be detected by Northern blotting, which will enable us to monitor the involvement of these ribonucleases in their maturation. After identifying ribonuclease candidates through Northern blot analysis, we will evaluate their impact on the production of all tsRNAs using high-throughput sequencing, a method we have specifically optimized for this purpose. Another objective of this study is to characterize the molecular complexes associated with tsRNAs. To accomplish this, we optimized the purification of tRNA fragments, utilizing a method developed by our laboratory that employs modified oligonucleotides complementary to the tsRNAs. With this approach, we will identify tsRNA-specific interactors through mass spectrometry analysis and determine their roles in tsRNA function.

This new study aims to understand how tsRNAs function under normal conditions, providing essential insights into a more comprehensive understanding of cellular function in animals. The initial findings of this research will be presented at this meeting.

453A Transcriptional interactions between host and nested genes during *C. elegans* development

Fabien Soulavie[1], Carole Couillault[1], Mateo Melki[2], Khulganaa Buyannemekh[1], Cristina Vico Cantero[1], Antoine Barriere[1], Paul Villoutreix[2], Vincent Bertrand[11]IBDM, Aix-Marseille University, [2]MMG, Aix-Marseille University

Embryonic development relies on tightly controlled gene expression. In this study, we analyzed the transcriptional effect during *C. elegans* development of a striking genomic topology, the opposite nested configuration, where a gene is located in an intron of a host gene in opposite direction. Using CRISPR genome engineering and single molecule FISH, we characterized the regulatory interactions between *ceh-10*, a transcription factor gene involved in neuronal specification, and its host gene *polq-1*, a DNA repair enzyme, showing that the nested gene induces expression of its host in neurons. Extending our analysis to the hundreds of protein coding genes in opposite nested configuration and using single cell RNA-seq data covering *C. elegans* embryogenesis, we observed that coexpression between nested and host genes is relatively common especially in cells positive for the nested gene. Finally, we are currently analyzing how coexpression of nested and host genes affects their transcriptional dynamics using two methods for *in vivo* imaging of transcription: the Argonaute NRDE-3 method and the MS2/PP7 method. Our study illustrates how the presence of a nested gene can influence expression of its host.

454A Evolution of the chromosomes of Nematoda and the limits of the Nigon element model

Mark Blaxter, Lewis Stevens, Erna King, Manuela Keininger, Martha Mulongo Wellcome Sanger Institute

Six years ago, we proposed a model for the chromosomal organisation of the last common ancestor of nematodes in order Rhabditida (Tandonent et al 2019 DOI: 10.1534/g3.119.0011). We called these seven ancestral linkage groups (ALGs) Nigon elements, and presented a model of the origins of the chromosomally-complete genome sequences of a small number of extant species through processes of fission and fusion-with-mixing. We showed that the Caenorhabditis X chromosome was formed from a fusion of Nigon N and Nigon X, but that only the Nigon X component tracked the X chromosome across species. We now have a hundred chromosomally complete genomes sampled across the phylogenetic diversity of Nematoda, and also genomes for species in Nematomorpha, the sister phylum. The Nigon model continues to effectively describe rhabditid nematode genomes. Through deeper sampling in Rhabditina we find that Nigon element boundaries in fused sex chromosomes are associated with sites of programmed DNA elimination. We identify species that have maintained the seven ancestral chromosomes, including an unfused, Nigon X-only sex chromosome. We have traced the origins of Nigon elements defined in Rhabditida to deeper within class Chromadorea, but find that the Nigon model does not describe the predicted ALG of the last common ancestor of Nematoda. The identity of the sex chromosome is different in these non-rhabditid nematodes, and the XY heteromorphic sex chromosomes of Nematomorpha do not carry genes orthologous to those that define the Nigon X.

455A Investigating the transcriptional regulation of autophagy upon heat stress and aging

Michelle Brown[1], Tatiana Moreno[2], Caroline Kumsta[1]1Sanford Burnham Prebys Medical Discovery Institute, [2]Sanford Burnham Prebys Graduate School of Biomedical Sciences

Autophagy, a cellular recycling process that degrades waste, is critical for maintaining homeostasis. Conversely, dysregulation and decline of autophagy with age leads to the accumulation of damaged cellular components and age-related disease proliferation. In *C. elegans*, the master transcription factor HLH-30, the homolog of TFEB in mammals, regulates autophagy gene expression and is activated by stress, including mild "hormetic" heat shock (hHS), which induces HLH-30/TFEB nuclear translocation. We have found that the nuclear translocation capacity of HLH-30/TFEB declines with age, but that an early-life hHS improves its nuclear translocation capacity with age. However, the mechanisms underlying HLH-30/TFEB regulation during stress and aging remain unclear.

HLH-30/TFEB activity is known to be regulated by post-translational modifications, including phosphorylation. Under nutrient stress, dephosphorylation facilitates TFEB's nuclear import, while AMPK-mediated phosphorylation activates TFEB. To investigate whether similar mechanisms regulate HLH-30 in response to heat shock and aging, we are performing structure-function studies to identify post-translational modifications and critical protein regions involved in stress-dependent activation. Additionally, we are analyzing HLH-30 expression at different temperatures, across age, and in response to hHS using Western blot to determine whether age-related declines in autophagy are due to reduced HLH-30 expression. We also aim to assess whether hHS alters HLH-30 expression levels in an age-dependent manner, potentially contributing to the observed improvements in autophagy with early-life hHS. Furthermore, a genome-wide RNAi screen aims to identify key regulators of HLH-30/TFEB function in both heat shock and aging contexts.

By elucidating the regulatory mechanisms of HLH-30/TFEB in response to heat stress and aging, our findings will provide critical insights into how stress-adaptive interventions, such as hormetic heat shock, can enhance autophagy and mitigate age-related decline.

456A Bio-ChIP reveals tissue-specific genome distribution of histone variants in *C. elegans*

Idris Selman Bulut[1], Alexander Blume[2], Sevinc Ercan[3], Baris Tursun[1]1Biology, Hamburg University, [2]BIMSB, MDC, [3]Biology, New York University

Chromatin occupancy by epigenetic regulators and histone proteins plays a key role in controlling spatial and temporal gene expression. Many chromatin-binding proteins have pleiotropic functions and are often expressed broadly in all tissues. Analyzing the tissue-specific occupancy of these factors by chromatin immunoprecipitation with sequencing (ChIP- Seq) is of particular interest and helps to dissect regulatory programs driving cell- and tissue-specific processes. The conserved histone variants H2A.Z and H3.3 have effects on gene regulation but are not well characterized with regard to context dependency in specific tissues. We are using an *in vivo* biotinylation-based ChIP-seq procedure, which we termed Bio-ChIP, to assess the distribution of H2A.Z and H3.3 in a tissue-specific manner. Bio-ChIP does not require cell sorting and overexpressing of the target histone proteins.

Applying Bio-ChIP to H2A.Z and H3.3 in *C. elegans* demonstrates superior efficiency compared to classical whole worm mixed-tissue ChIP with antibodies. Our comparison reveals that the latter delivers biased results for certain tissues.

Bio-ChIP demonstrates that H2A.Z has tissue-specific genome distribution but at a significantly lower rate than H3.3. The genome binding profile of H3.3 in intestine, neurons, and the germline show significant variation, suggesting involvement in tissue-specific gene regulation. Interestingly, specific occupancy of numerous genomic loci contain piRNAs in the germline but also in the soma. Additionally, we find that H3.3 delocalizes from intestinal genes in aged intestine, indicating genome distribution dynamics upon aging.

Overall, Bio-ChIP of histone proteins for mapping tissue-specific genome distribution is a powerful application that helps us better understand chromatin regulation in a context-dependent manner.

457A Investigating a novel candidate gene in PVD dendrite morphogenesis

Zipporiah K Bush[1], Nelson Ramirez-Suarez[2], Meera Trivedi[2], Leo Tang[2], Hannes Buelow[21]Genetics, Albert Einstein College of Medicine, [2]Albert Einstein College of Medicine

Humans and animals alike rely on dendrites of sensory neurons to perceive environmental stimuli. Dendrite development requires a delicate balance of growth, branching, self-avoidance, and pruning. Dysregulation during any of these stages can lead to deformed dendrites, which underlie human diseases such as Tuberous Sclerosis and Fragile X Syndrome. By using *C. elegans* as a model, we study the pair of somatosensory neurons known as PVD, which are known for their intricate, menorah-like dendritic branching. This branching is tightly regulated by the menorin complex, with factors present in the epidermis, muscle, and PVD itself. It has been shown that *kex-2/subtilisin-like proprotein convertase 1* (*kpc-1*) serves as a negative regulator to the menorin signaling complex, but whether it has potential substrates besides itself during autocatalytic cleavage remains unclear. In a modifier screen of a *kpc-1* hypomorph, we identified a mutant allele (*dz217*) phenotype closely resembling that of *kpc-1* null mutants yet distinct from single mutants of menorin complex factors. Based on SNP mapping, we have located a series of polymorphisms in a region that may be responsible for the *dz217* phenotype. Thus, the current project aims to (1) identify and characterize the gene affected by the *dz217* mutant, (2) determine where this novel gene functions, and (3) investigate its function in the context of PVD dendrite patterning.

458A N-terminal IDR and small RNA binding regulate HRDE-1 nuclear import

Maria R Bustos, Carolyn Phillips Molecular and Computational Biology, University of Southern California

Nuclear RNA interference (RNAi) is an essential gene regulatory pathway that is vital for gametogenesis, germline stability, and heterochromatin formation. *C. elegans* has two nuclear Argonaute proteins, HRDE-1 and NRDE-3, which are integral to the nuclear RNAi pathway. The two nuclear Argonaute proteins load small interfering RNAs (siRNAs), interact with nascent transcripts through base pairing, and recruit the downstream silencing factors, NRDE-1, NRDE-2, and NRDE-4. Gene silencing is then established by inhibiting RNA polymerase II elongation or through the deposition of repressive chromatin modifications that form heterochromatin. The HRDE-1-mediated silencing events are inherited across multiple generations and are vital for transgenerational fertility. Argonaute proteins must enter the nucleus to carry out transcriptional gene silencing. However, the mechanism mediating Argonaute nuclear import remains largely unknown.

We have shown that HRDE-1 localizes to perinuclear germ granules to load the correct siRNAs before entering the nucleus to mediate transcriptional gene silencing. We also find that HRDE-1 nuclear import is impaired when the siRNA binding pocket is mutated, in which case HRDE-1 localizes to germ granules and the cytoplasm. In this study, we demonstrate how siRNA binding might regulate HRDE-1 nuclear import by exposing the nuclear localization signal. Furthermore, we show that the HRDE-1 N-terminal intrinsically disordered region is important for siRNA binding and nuclear import. Moreover, we identified the importin proteins IMA-3 and IMB-1 as HRDE-1 nuclear import factors, implicating the importin α/β pathway in mediating Argonaute nuclear import. Taken together, these results provide critical insight into the regulation and mechanism of Argonaute nuclear import. Additionally, our results demonstrate the potential significance of the N-terminal domain on Argonaute siRNA loading in *C. elegans*.

459A Collaboration between two layers of gene repression establishes and maintains cell fate

Megan Butler, Luisa Cochella Molecular Biology and Genetics, Johns Hopkins University School of Medicine

Cell identity is determined by transcriptional programs that require gene activation, but also specific gene repression. Repression comes in two main forms: a heterochromatic environment that blocks activators from a given region of the genome, and sequence-specific transcription factors (TFs) that recruit co-repressors which lead to gene silencing. How each repressive layer contributes to cell identity is not well understood because their effects are difficult to detangle in most complex systems and in genome-wide approaches. One well-studied paradigm for cell fate decisions is the left/right asymmetry in the *Caenorhabditis elegans* ASE neurons. ASEL (left) and ASER (right) are bilaterally symmetric sensory neurons specified by the same sequence-specific TF, CHE-1, which activates all common ASE genes. However, a specific target, *lsy-6*, is only activated by CHE-1 in the left neuron and not in the right. *lsy-6* is a microRNA that acts as a genetic switch, leading to molecular and functional L/R asymmetry of the ASE neurons. Previous work in our lab found that a repressive state of *lsy-6* in ASER is established during early embryogenesis. We hypothesize that repressive chromatin is involved because deletion of HP1 causes early, low-penetrance de-repression of *lsy-6* in ASER. Intriguingly, heterochromatin appears insufficient for repression maintenance since deletion of a particular sequence motif in the *lsy-6* promoter results in later, robust de-repression of *lsy-6* in ASER. This motif is similar to the binding site for a family of C2H2 zinc finger TFs, so such a TF may bind to these motifs in the *lsy-6* promoter and recruit co-repressors to keep *lsy-6* off for the life of the worm. From screening the co-repressor complexes in *C. elegans* by RNAi, we found the NuRD complex nucleosome remodeler subunit *let-418* is essential for later repression of *lsy-6* in ASER. Therefore, we propose a model where repressive chromatin and a sequence-specific TF with co-repressors collaborate to specify and maintain cell identity.

460A The GAGA Factor, EOR-1, is a potential pioneer factor in *C. elegans*

Sylvia Cevallos, Sofia Doune-Bosch, Kayleigh Davies, Christopher Anosike, Abigail Nasse, Dagmawi Lulseged, Amanda Fuenzalida, Olivia Howard, Jada Coffey, Will Schuckenbrock, Deborah M Thurtle-Schmidt Biology, Davidson College

How transcription factors navigate the chromatin landscape to precisely coordinate cell-specific gene regulatory networks is not well understood. To investigate this precise transcriptional control, we profiled the genomic binding landscape of a well-conserved transcription factor in *C. elegans*, NHR-25. Interestingly, in addition to the NR5A1 motif, which NHR-25 binds, we also identified a GAGA motif enriched at NHR-25 binding sites. In *C. elegans*, the GAGA motif is bound by the conserved BTB/zinc-finger transcription factor EOR-1. To investigate a possible interaction between *nhr-25* and *eor-1*, we performed phenotypic assays which showed that *eor-1* mutants have a developmental delay and enhance *nhr-25* mutant egg-laying defects. Thus, like *nhr-25, eor-1* is an important developmental regulator and genetically interacts with *nhr-25*. To molecularly characterize shared regulated genes by *nhr-25* and *eor-1*, we performed RNA-seq of *nhr-25* and *eor-1* mutants, identifying a shared transcriptome of stress and metabolism related genes. Previous research indicates that EOR-1 interacts with chromatin remodelers (Shinkai *et al.* 2018) and the *Drosophila* ortholog is classified as a pioneer factor (Gaskill *et al.* 2021). Thus, we hypothesize that EOR-1 acts as a pioneer factor necessary for NHR-25 binding in *C. elegans*. To interrogate this hypothesis, we are characterizing NHR-25 binding that is dependent on EOR-1, through performing CUT & RUN on NHR-25 in an *eor-1* mutant. These data will be correlated with differential accessibility sites in an *eor-1* mutant through ATAC-seq revealing EOR-1 dependent chromatin accessibility. Through these genetic and genomic analyses, we show that EOR-1 is a key developmental regulator and are identifying a subset of NHR-25 regulated genes dependent on a putative pioneer factor, EOR-1.

461A Understanding the effect of miR-1 mediated repression on V-ATPase assembly in muscle

Lo-Yu Chang[1,2], Luisa Cochella[11]Department of Molecular Biology and Genetics, Johns Hopkins University, [2]Biochemistry, Cellular and Molecular Biology Graduate Program, Johns Hopkins University

MicroRNAs (miRNAs) are a class of short, non-protein-coding regulatory RNAs that post-transcriptionally repress gene expression. A subset of miRNAs is highly expressed specifically in muscle cells and is collectively known as myomiRs. One of the most abundant myomiRs is miR-1, whose sequence and muscle-specific expression pattern are conserved across Bilateria. Various prediction tools had suggested that multiple subunits of vacuolar-type ATPase (V-ATPase) are conserved targets of miR-1 throughout Bilateria—a finding that our lab recently validated experimentally in *C. elegans* and *Drosophila* (Gutierrez-Perez et al, 2021). Intriguingly, in *C. elegans*, the effect of knocking out miR-1 is the loss of function of the V-ATPase (LoF), despite the fact that multiple subunits are de-repressed at the mRNA level. The mechanism by which de-repression of individual V-ATPase subunits causes LoF of the complex remains to be explored and is limited by our ability to visualize V-ATPase proteins or use biochemical tools that are challenging in *C. elegans*.

Here, I will present our efforts on two approaches to understand the effect of miR-1 on the V-ATPase.

1) We are taking advantage of a split-fluorescence system (Goudeau et al. 2021) to achieve tissue-specific labeling of endogenously tagged VHA-13 (ATP6V1A) in body wall muscle (BWM). This has revealed that the level of VHA-13 protein is higher in miR-1 knockout muscle cells. We are developing this strategy to monitor expression of other subunits and explore their localization and V-ATPase assembly status.

2) As miR-1 is highly conserved and is predicted to target multiple V-ATPase subunits in vertebrates, we leveraged an *in vitro* murine myoblast culture system, C2C12, to experimentally validate target conservation and develop a homogeneous cell system for biochemistry. Using an optimized CRISPR-enCas12a genetic editing system, we successfully generated a triple knockout (tKO) for the *mir-1* family in C2C12 cells. ATP6V1A (VHA-13), ATP6V1B2 (VHA-12), and ATP6V1D (VHA-14) were all de-repressed in the tKO C2C12 cells, which exhibited myotube fusion defects and a vacuole formation phenotype.

Overall, our work suggests that multiple V-ATPase subunits may be conserved targets of miR-1, which is essential for proper muscle physiology.

462A Single-cell RNA sequencing of *C. elegans mpk-1*/ERK mutants

Armi Manharbhai Chaudhari[1], Patrick Narbonne[21]Department of medical biology, Universite du Quebec A Tois-Rivieres, [2]Department of medical biology, University of Quebec trois riveres

In *Caenorhabditis elegans*, the *mpk-1* gene encodes a mitogen-activated protein kinase (MAPK) that plays a crucial role in various cellular processes. MPK-1 promotes germline stem cell proliferation cell non-autonomously from somatic tissues and is important, both autonomously and non-autonomously, for gamete differentiation. It further influences fate determination in multiple tissues, ensuring proper organogenesis and cellular homeostasis. Given its functional similarity to human ERK/MAPK proteins, studying MPK-1 in *C. elegans* may provide valuable insights into disease mechanisms, particularly those related to cancer and developmental disorders. Single-cell RNA sequencing (scRNA-seq) data of *mpk-1(ø)* mutants in *C. elegans* could provide valuable insights into its role in cellular differentiation, signaling, and stem cell regulation within the different tissues where it is required. Furthermore, scRNA-seq can help uncover co-expressed genes and downstream targets of MPK-1, shedding light on its regulatory networks. We therefore wish to use the well-characterized *mpk-1(ga117)* null mutant for scRNA-seq. However, the fully penetrant sterility of this allele made it particularly challenging to isolate a pure population of *mpk-1(ga117)* homozygote adults. For propagation, these mutants are balanced by the *qC1[dpy-19(e1259) glp-1(q339) qIs26[rol-6(su1006)gf; Plag-2::GFP]]III* rearrangement, while performing scRNA-seq on this balanced strain would have introduced a major limitation as the presence of a high proportion of *mpk-1(+/-)* cells would reduce detection of *mpk-1(ø)* cells and their specific transcriptional changes, potentially masking key regulatory insights. Additionally, low transcript counts from mutant cells could prevent the detection of specific cell clusters and reduce the resolution of lineage-specific effects. Despite these challenges, optimizing sequencing depth and employing computational approaches to enrich *mpk-1*-deficient populations may improve data quality and enhance our understanding of *mpk-1* function at the single-cell level. Yet, we developed a FACS based protocol to isolate GFP negative *mpk-1(ø)* eggs and to perform the single cell isolation on a synchronized homozygous *mpk-1(ø)* population. We therefore hope to obtain a very high ratio of *mpk-1(ø)* cells. Given its evolutionary conservation, studying MPK-1 at the single-cell level can enhance our understanding of similar MAPK signaling pathways in humans, with implications for developmental biology and disease research.

463A Chromatin factor MRG-1 interacts with multiple chromatin-modifying complexes to regulate germline gene expression

Mindy M Clark[1], Margaret R Starostik[2], Katrina Wang[1], Amelia F Alessi[1], Victoria N Murphy[1], Jessica A Kirshner[1], John K Kim[11]Biology, Johns Hopkins University, [2]Johns Hopkins University

Maintaining chromatin architecture in the germline is essential for proper gene expression and germ cell integrity. Our work investigates how chromatin-associated factors in the *C. elegans* maternal germline regulate the heterochromatin-euchromatin landscape to preserve germ cell identity. A key factor in this process is MRG-1, an essential H3K36me3 reader whose loss results in gene misregulation, germ cell fate loss, and ultimately sterility. As expected, ChIP-seq analysis of purified germline nuclei shows that MRG-1 binds along the gene bodies of actively transcribed genes in the adult gonad, partially mirroring the distribution of the euchromatin-associated histone mark H3K36me3. However, MRG-1 is also particularly enriched at the transcriptional starts sites (TSSs) of these genes, suggesting an unexpected remodeling event specific to the adult germline and early embryonic development. We hypothesize that MRG-1 is recruited to germline-active gene bodies through its canonical interaction with H3K36me3, while its enrichment at TSSs may facilitate the recruitment or stabilization of transcriptional activating complexes required for proper gene expression. Supporting this model, mass spectrometry analysis of immunopurified MRG-1 from germline nuclei reveals strong interactions with two histone acetyltransferase complexes and H3K4 methyl-associated factors. Like H3K36me3 and MRG-1, multiple of these interactors are also required for proper germline gene expression. Ongoing investigations aim to define how MRG-1 and its associated complexes collaborate to establish a robust, heritable gene expression program that maintains germline identity across generations.

464A Enhanced RNAi Vectors for Potent Multi-Gene Silencing in *C. elegans*

Sophia Cofone, Daniel Shaw, Svilen Kolev, Mirjam Kreutz, Sree Harsha Zenitha Vadakattu, Isabella Cuthbertson, Naadia Rashid, Javier Apfeld, Erel Levine Northeastern University

Despite the widespread use of RNA interference (RNAi) in C. elegans, consistent, simultaneous suppression of multiple genes remains challenging. We present an optimized RNAi vector and approach that enhance and streamline multi-gene silencing experiments. Our vector incorporates modified transcriptional elements for improved silencing efficiency and Golden Gate sites for simplified cloning of single or multiple RNAi targets. Further, we provide a companion plasmid that enables co-transformation of two stable RNAi constructs, allowing for an economical mix-and-match practice. Our approach enables efficient silencing of up to five genes, including both endogenous genes and integrated fluorescent proteins. Compared to current reagents, the new vector yields phenotypes of equal or greater strength and penetrance. We find consistently high efficiency across delivery methods for multi-gene suppression, including bacterial mixing, co-transformation, and a single vector with multiple targets. This updated vector system and approach simplify multi-gene knockdowns, enabling genetic interaction studies and large-scale screens.

465A Oleic acid increases *C. elegans* metabolism and fecundity by altering DAF-12 activity

Frances V Compere[1], Alexandra M Nichitean[2], Sarah E Hall[1]1Biology, Syracuse University, [2]Genetics, Cellular Biology, and Development, University of Minnesota

Early life stress during critical periods in development can lead to phenotypic plasticity in adult animals. We use *C. elegans* dauer formation to investigate the mechanisms regulating phenotypic consequences of early-life starvation. In favorable conditions, L1 larvae develop continuously to become reproductive adults (controls). In contrast, nutritional stress promotes entry of L1 larvae into the stress-resistant dauer stage. When conditions improve, dauers will resume their development to become reproductive, postdauer adults (PD). We have previously shown that PD adults have downregulation of germline-expressed genes and upregulation of genes associated with fatty acid metabolism, correlating with decreased fecundity and decreased stored intestinal lipids compared to control adults. The decreased fecundity was dependent on the DAF-12 steroid signaling pathway and Δ9 desaturases, *fat-5, fat-6,* and *fat-7*. To elucidate the connection between the metabolic and reproductive phenotypes, we performed brood size assays with PD animals whose OP50 food was supplemented with various fatty acids. Only oleic acid (OA) and vaccenic acid increased the fecundity of adult animals. However, only the OA increase in fecundity was dependent on DAF-12, suggesting that different fatty acids may act through distinct pathways to regulate reproduction. Given that OA also requires FAT-7 to increase fecundity, we next used a *fat-7::gfp* transcriptional reporter to investigate whether DAF-12 regulates *fat-7*. We found that *fat-7* expression increases in PD adults compared to controls as shown by RNA-seq in a DAF-12-independent manner. However, OA dietary supplementation further increased *fat-7* in both populations in a DAF-12-dependent manner, demonstrating that OA can alter the activity of DAF-12. DAF-12 is a nuclear hormone receptor (NHR), which are known to modulate their activity in response to environmental cues through working with other NHRs. Using a candidate gene approach, we identified two additional NHRs, NHR-13 and NHR-66, that are required for the increased fecundity with OA dietary supplementation. We are currently testing whether DAF-12 binds with either of these NHRs in the context of OA to alter its activity. Together, our results demonstrate that metabolism and reproduction are modulated by the complex activity of DAF-12 and other NHRs, which serve as sensors of an animal's metabolic state and regulate their activity accordingly.

466A Identification of a Genetic Suppressor of Antimorphic alg-1 Mutations

Heather Crawshaw, Jeff Medley, Anna Zinovyeva Biology, Kansas State University

Regulation of gene expression is a fundamental process that enables diverse cellular functions by controlling the magnitude, timing, and location of different gene activities. Proper gene regulation is essential for normal development, while disruptions in this process are associated with several cancers and developmental disorders. Among the key regulators of gene expression are microRNAs (miRNAs), which are a class of non-coding RNAs that play a crucial role in post-transcriptional gene silencing by repressing the activity of target genes. During miRNA biogenesis, miRNAs are loaded into Argonaute proteins to form a functional complex that represses specific target transcripts based on complementary pairing of the miRNA loaded in the Argonaute with the target. The *let-7* miRNA is evolutionarily conserved across metazoans and, in the nematode *Caenorhabditis elegans,* is absolutely required for animal development. In *C. elegans*, the Argonaute ALG-1 serves as one of two primary mediators of the miRNA pathway and associates with the let-7 miRNA. Loss of *let-7* or *alg-1* function results in aberrant gene regulation of let-7 target genes, and failure to progress through normal developmental stages. Here, we identified a spontaneous suppressor mutation that restores proper development and stage-specific gene expression in antimorphic *alg-1(ma202)* and *alg-1(ma192)* mutants. Our genetic mapping data indicates that the suppressor gene is located on the left side of Chromosome I on an interval comprising six potential candidate variants identified through whole genome sequencing. We are currently performing CRISPR/Cas9 genome editing of these variants to determine the molecular identify of the *alg-1* suppressor. Further characterization of the *alg-1* suppressor will shed light on the mechanisms that fine-tune miRNA-mediated gene regulation during animal development.

467A Uncovering the role of heterochromatin spatial architecture in environmental stress response

Yuri D´Alessio, Fernanda Rezende Pabst, Johanna Hornung, Daphne S. Cabianca Institute of Functional Epigenetics, Helmholtz Zentrum Munich

Sequestration of heterochromatin, the transcriptionally repressed portion of the genome, at the nuclear periphery is an evolutionarily conserved, active process that increases with cell differentiation and it is disrupted in aging and cancer. Yet, despite its relevance, the function of the spatial organization of the genome remains largely unknown due to the inability to perturb it while leaving other nuclear processes unaltered.

C. elegans is the only known multicellular organism where a heterochromatin anchoring-dedicated factor has been identified: CEC-4. CEC-4 is a highly specific H3K9me reader which localizes at the nuclear envelope where it anchors H3K9me-marked chromatin. Indeed, the loss of CEC-4, as well as the lack of H3K9me, leads to heterochromatin detachment from the nuclear periphery in embryos. However, while the absence of H3K9me leads to the misexpression of hundreds of genes, *cec-4* mutants exhibit only minor changes, challenging the idea that subnuclear positioning regulates transcription. Here, we used mutations in *cec-4* to test whether perinuclear anchoring of heterochromatin plays a role in responding to unscheduled transcriptional stimuli triggered by environmental stress. Our data suggest that CEC-4 has a negligible role on the global transcriptional regulation during the response to heat or cold stress in L1 larvae, a conclusion supported also by animal survival. However, interestingly, when animals at a later developmental stage (L4s) are exposed to the same stressors, the survival of *cec-4* mutants is higher compared to that of wild type worms—a phenotype mediated by the loss of CEC-4's histone methyl-binding activity. These results suggest that CEC-4 gains a novel function after the L1 stage. Accordingly, live imaging of a functional, fluorescently tagged CEC-4 revealed that, during larval development, the enrichment of CEC-4 at the nuclear periphery shifts from being H3K9me-independent—until the L1 stage, to partially H3K9me-dependent, with CEC-4 partially localizing in the nucleolus in absence of H3K9me.

Ongoing experiments aim at exploring the role of CEC-4 and its subnuclear localization in regulating gene expression in late larvae and adults both in standard conditions as well as in response to temperature shocks.

468A Understanding the role of small RNAs and RNA interference components in DNA damage repair

Yakshi Dabas, Lamia Wahba Laboratory of Non-Canonical Modes of Inheritance, The Rockefeller University

Stable transmission of genetic information across generations is critical for species survival. The propagation of DNA across cell divisions is challenged by multiple endogenous and exogenous sources that induce deleterious lesions such as double-strand breaks. To counteract this damage, a variety of DNA repair and checkpoint pathways have evolved, forming a complex signaling cascade that detects DNA damage, and recruits a set of specialized proteins to facilitate repair. Although much of the DNA repair machinery has been identified and characterized at a molecular level, several aspects of the DNA damage response (DDR) pathway remain to be fully elucidated, limiting our understanding of how DDR is orchestrated in complex genomic and cellular environments. One such poorly characterized factor is RNA. Recent work in human cells, plants, fungi, and *Drosophila* has implicated a role for small RNAs and the RNA interference (RNAi) machinery in facilitating DNA repair. Despite intriguing evidence that the DDR pathway links RNAi and DNA repair, mechanistic insight on the function(s) for the small RNAs themselves in DDR remain elusive. By profiling the small-RNAome of *C. elegans* upon damage, we found a significant 5.56-fold increase in antisense small RNAs against ribosomal RNA genes. We also found that *C. elegans* strains lacking components of the endogenous small interfering RNA (endo-siRNA) and Nuclear RNAi pathway exhibit increased DNA damage sensitivity analogous to DNA repair mutants. We hypothesize that damage-induced small RNAs represent a regulatory response to DNA damage, and RNAi mutants that fail to generate these small RNAs are unable to repair their DNA efficiently. The enrichment of antisense ribosomal siRNAs could be triggered by the damage at the ribosomal DNA(rDNA) locus itself, wherein small RNAs generated at the site of break help facilitate its repair. Indeed, links between ribosomal siRNAs, and rDNA copy number maintenance have been identified in organisms such as *Neurospora*. Alternatively, antisense ribosomal siRNAs could represent a potential checkpoint system that gets activated to modulate translation in response to DNA damage. Understanding the role of small RNAs in context of DNA repair will reveal exciting avenues by which small RNAs can mediate genome stability.

469A Investigating CEC-4 Localization and Function in Heterochromatin Anchoring

Julia A D'Arca, Steven Yenglin, Györgyi Csankovszki MCDB, The University of Michigan

The nuclear lamina plays a critical role in organizing chromatin structure. CEC-4 is a nuclear laminar tethering protein which binds methylated H3K9 residues and recruits heterochromatin to the nuclear envelope. This interaction is essential for chromosome organization, and compaction, and it impacts cell fate specification in development and regulation of the X chromosomes during dosage compensation. Our observations of a *cec4::mCherry* transgene indicates that the protein localizes to the nuclear lamina, consistent with its proposed role in heterochromatin anchoring. CEC-4 forms a ring at the nuclear periphery at all embryonic stages. To further investigate CEC-4's localization and function, we are developing various tagged versions of CEC-4 to study its interactions at a biochemical level. We initially inserted a 3XFLAG tag at the C-terminus, the same place as the mCherry tag in the transgene, but at the endogenous locus. Unlike CEC-4::mCherry, CEC-4::3XFLAG was localized throughout the nucleus. We then inserted a flexible linker between CEC-4 and the FLAG tag, but the CEC-4::linker::3xFLAG was still diffusely localized throughout the nucleus. Ongoing experiments focus on characterizing the localization of CEC-4::mCherry and CEC-4::3X*FLAG* across different nuclear compartments to determine why the addition of FLAG tag affects its localization, and whether the disrupted localization affects CEC-4's tethering ability. These studies will provide critical insights into the molecular mechanisms governing chromatin organization at the nuclear envelope. By dissecting CEC-4's role in heterochromatin anchoring, this work contributes to our broader understanding of nuclear architecture and its implications in genome regulation.

470A HRDE-1 and PRG-1 are required for intergenerational inheritance of starvation memory in postdauer progeny

Savannah L. Davis[1], Maria C. Ow[2], Sarah Hall[11]Biology, Syracuse University, [2]Syracuse University

Human famine studies have shown that early life exposure to starvation can significantly impact an individual's health later in life. For example, the Dutch Hunger Winter famine demonstrated that nutritional stress *in utero* can lead to a higher risk for metabolic disorders, cardiovascular disease, and obesity in adulthood. Additionally, the childrenand grandchildren of these individuals were also more likely to develop obesity, glucose intolerance, and cardiovascular disease, suggesting that a "starvation memory" can be inherited across generations. We have developed *C. elegans* as a model organism to investigate the mechanisms that coordinate a heritable memory of starvation. In favorable conditions, *C. elegans* animals will pass through four larval molts to become reproductive adults (controls). In contrast, starvation stress triggers early-stage *C. elegans* larvae to enter a stress-resistant, non-feeding stage called dauer diapause. When the stress conditions alleviate, dauer larvae will resume development to become reproductive, postdauer adults (PD). We have demonstrated previously that PD adults exhibit higher expression of lipid metabolism genes and lower expression of genes regulating reproductive development compared to control adults.Furthermore, PD adults showed decreased intestinal lipid storage and increased embryonic lipid storage as a response to early life starvation. Interestingly, the increased embryonic lipid storage in developing F1 embryos correlated with increased levels of intestinal lipid storage in the adult F1 progeny, which was "reset" to control levels in the F2 embryos and adults. Analysis of oil red O staining in candidate mutant strains revealed that HRDE-1/WAGO-9 was required for inheritance of the starvation memory in PD F1 progeny, while PRG-1/PIWIwas required to restore the lipid storage phenotype to control levels in the F2 progeny. Currently, we are using sRNA-seq to identify the gene targets of HRDE-1 and PRG-1 that are causal to the lipid storage phenotypes described above. Together, our work has demonstrated that *C. elegans* PD adults retain a starvation memory that is intergenerationally inherited via RNAi pathways, and that this system can serve as a model for nutritional stress in humans.

471A Parental age influences mitochondria-related gene expression to affect early fecundity

Elizabeth Hogan, Ava Adriani, Diana Chirila, Madison DePeri, Amy Webster Florida State University

Parental age impacts a variety of progeny phenotypes in *C. elegans*, including developmental rate, size, and fecundity. While vitellogenin provisioning has been implicated to explain a variety of parental age-induced phenotypes, differences in vitellogenin provisioning do not explain differences in progeny fecundity. We previously scored early fecundity in the progeny of day 1 and day 3 adults and performed single-worm mRNA-seq on each worm measured. Interestingly, we found parental age caused robust changes in genes expressed from the nuclear genome and involved in a variety of mitochondrial processes. Because we directly measured early fecundity in each worm sequenced, we also determined that these mitochondrial genes are positively associated with early fecundity. To determine whether these genes causally affect early fecundity, we knocked them down using RNA interference. We found that genes involved in multiple mitochondrial processes are required for early fecundity. Collectively, our results provide preliminary evidence that parental age causes differences in mitochondria-related gene regulation that are passed on to progeny to impact fecundity.

472A Dynamic regulation of miRNA-mediated silencing through stage-specific miRISC composition

Joseph Dixon[1], Amelia Alessi[2], Himani Galagali[2], Lars Benner[2], Youngyong Park[2], Jolene Diedrich[3], John R. Yates[3], John K Kim[21]Johns Hopkins, [2]Johns Hopkins University, [3]Scripps Institute

MicroRNAs (miRNAs) trigger gene silencing by partially base pairing with the 3' UTRs of target transcripts, leading to mRNA degradation, translational repression, or both. While mechanisms of target mRNA degradation are well characterized, the process by which miRNAs mediate translational repression remains poorly understood. Recent work demonstrates that miRISC composition can have profound effects on its mode of silencing. In the soma, the miRISC cofactor AIN-1 (GW182 in humans) promotes target mRNA degradation, whereas in the germline, miRISC associates with GLH-1 to repress translation without affecting mRNA stability. These findings prompted our lab to investigate stage-specific miRISC interactors by mass spectrometry analysis of immunopurified miRISC in C. elegans. In vivo validation of miRNA phenotypes following RNAi knockdown of these interactors has uncovered multiple candidate miRNA regulators, including L4-enriched factors RACK-1, ICD-1, and CEY-1. To further elucidate their role in miRISC-mediated regulation, our current work includes ribosome profiling to assess their impact on miRNA target translation. By integrating genetic, biochemical, and translational efficiency assays, we aim to provide insights into how miRISC composition dynamically regulates miRNA target silencing during development, ensuring robust developmental transitions

473A Transposable element and gene family evolution across Rhabditidae

Tori K Eggers, Jason Pienaar, Janna L Fierst Biological Sciences, Florida International University

The C-value paradox highlights the lack of correlation between genome size and organismal complexity. This paradox might be explained by repetitive DNA, particularly transposable elements (TEs), which are thought to drive genome expansion in many species and lead to the decline of self-fertile populations. In *Caenorhabditis,* it is known that self-fertile species have consistently smaller genomes than closely related outcrossing relatives. However, the mechanisms underlying genome size variation in nematodes remain unclear. Previous studies found little association between TE content and genome size, but many lacked power due to small sample sizes, poor genome assembly quality, and limited phylogenetic methods. In addition, past studies solely use TE content while ignoring TE dynamics and their interactions with other genomic features like genes. Here, we annotate genes, TEs, and ultra-conserved regions of approximately 70 chromosome-scale Rhabditidae genomes. Additionally, we conduct a multivariate phylogenetic comparative analysis between androdiecious and dioecious species, allowing for varying evolutionary optimums across the phylogenetic branches to investigate long-term drift, as well as short, transient dynamics of TE evolution.

474A Generating a stable PIWI Argonaute in the absence of piRNAs

Kimberly S Elicker, Lamia Wahba The Rockefeller University

Caenorhabditis elegans possesses multiple small RNA pathways that regulate gene expression, including microRNAs (miRNAs), endogenous small interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs). Among these, piRNAs function specifically in the germline, where they help maintain gene regulatory balance across generations. In *C. elegans*, piRNA biogenesis requires the transcription factor PRDE-1, and mature piRNAs are loaded into the PIWI Argonaute protein PRG-1. Loss of either *prde-1* or *prg-1* results in a progressive, heritable sterility known as the Mortal Germline (Mrt) phenotype. piRNAs are proposed to restrain the amplification of siRNAs, implying broader roles for PIWI in germline regulation. Whether these roles are mediated by specific piRNAs or by the PIWI protein itself remains unknown. Deciphering this has been challenging since the stability of both molecules is interdependent: piRNAs are rapidly degraded without PIWI, and PIWI is destabilized in the absence of piRNA loading. To test the piRNA-dependent and -independent functions of PIWI, we are generating a stabilized version of PIWI that persists in the absence of piRNAs. In *prde-1* null animals expressing GFP::PRG-1, we performed EMS mutagenesis and used flow cytometry to isolate mutants that retain GFP signal, indicating stabilization of unloaded PIWI. This approach aims to determine whether the Mrt phenotype results from the absence of piRNAs or from the loss of PIWI function itself, thereby uncoupling PIWI's piRNA-guided activity from potential piRNA-independent roles in germline maintenance.

475A Identification and validation of variants impacting pain sensitivity and morphine effectiveness in pediatric cancer patients

Graeme Ernest-Hoar[1,2], Erika N Scott[2,3], Jia He Zhang[1,2], Rena M Daniel[2,3], Colin JD Ross[2,4], Shahrad Rod Rassekh[2,3], Bruce C Carleton[2,3,5], Brock Grill[6,7,8], Catharine Rankin[9,10], Catrina M Loucks[1,2,31]Anesthesiology, Pharmacology, and Therapeutics, University of British Columbia, [2]BC Children's Hospital Research Institute, [3]Pediatrics, University of British Columbia, [4]Faculty of Pharmaceutical Sciences, University of British Columbia, [5]Pharmaceutical Outcomes Programme, BC Children's Hospital, [6]Pediatrics, University of Washington, [7]Pharmacology, University of Washington, [8]Center for Integrative Brain Research, Seattle Children's Hospital Research Institute, [9]Psychology, University of British Columbia, [10]Djavad Mowafaghian Centre for Brain Health

Background: Patients receiving chemotherapeutics are at a high risk of developing painful adverse reactions, with approximately 50% of surveyed cancer patients reporting such pain. Opioid analgesics, such as morphine, are commonly used to treat this pain, yet individual responses can vary substantially, limiting their effectiveness. This variability in pain and opioid responses is due, in part, to differences in genetic traits responsible for opioid biotransformation. Due to homology in many genes involved in pain/analgesia, Caenorhabditis elegans can effectively model this variability, thereby validating genetic associations.

Objectives: We aim to identify genetic variants influencing pain and opioid responses in pediatric oncology patients suffering from painful adverse reactions to chemotherapy, and validate identified genetic associations using C. elegans.

Methods: Genomic analyses will be conducted on a cohort of 1833 pediatric oncology patients recruited through the Canadian Pharmacogenomics Network for Drug Safety. Identified genes/variants will be validated by assessing C. elegans loss-of-function mutants for pain-related behaviours (using chemical avoidance and harsh touch assays) and opioid sensitivity (using assays for inhibited locomotion in the presence/absence of morphine). Results: We identified 1067 patients who experienced a painful adverse reaction to chemotherapy, of which 275 were treated with morphine for their pain. Preliminary analyses in 85 morphine-treated patients revealed that 30.6% experienced morphine ineffectiveness and that genetic variation in COMT is associated with adequate pain relief from morphine. Subsequent assays showed that C. elegans mutants for COMT (i.e., comt-4 mutants) paralyzed to a greater extent in morphine than wild-type animals. Given that COMT is involved in the catabolism of catecholamines (e.g., dopamine) that play important roles in pain sensitivity and morphine-based pain relief, additional assays are being conducted for dopamine-related genes also implicated in human pain/opioid phenotypes. Specifically, we are assessing C. elegans mutants for SLC6A2 (dat-1), TH (cat-2), and DRD3/DRD2 (dop-3), where preliminary assays of dat-1 mutants show enhanced morphine inhibited locomotion phenotypes. Conclusions: This study will inform the development of predictive genetic tests and accompanying clinical practice recommendations to help guide pain management and reduce the burden of pain in these patients.

476A Membrane Trafficking Pathways Regulating Exophergenesis in *Caenorhabditis elegans*

Mark Abbott, Sarah Farah, Ilija Melentijevic, Ryan Guasp, Monica Driscoll Genetics, Rutgers

To maintain proteostasis within an organism, neurons utilize autophagy, proteasome-mediated degradation, and other specialized processes to remove misfolded and aggregated proteins from within an organism's cellular network. While experiencing high proteostress, *Caenorhabditis elegans* neurons exhibit the ability to expel toxic protein aggregates and organelles in vesicles to maintain proteostasis in membrane-bound vesicles called exophers. The genetic process of creating exophers, or exopher-genesis, is largely unknown. A high-throughput, whole-genome RNA interference screen was conducted to identify possible modulators of the process. To quantify the results of the RNAi screen, images were captured using a high content imager; consequently, exopher production rates were calculated to identify genes of interest. As a result of the assay, 91 possible genes that may contribute to exopher-genesis in *C. elegans* strain ZB5786. My project will specifically focus on identifying membrane trafficking genes and studying their function.

477A Transcriptomic analysis identifies mechanisms of action of coffee diterpenes in *Caenorhabditis elegans*

Renalison Farias Pereira Biological Sciences, Kean University

Coffee diterpenes (kahweol and cafestol) are potential bioactive compounds found in the lipophilic fraction of coffee seeds that may help improve the health span of *Caenorhabditis elegans*. Previously, coffee diterpenes have been shown to affect lipid metabolism via distinct mechanisms: kahweol by reducing food intake, and cafestol by increasing energy expenditure. This study aims to compare the transcriptomic profiles of nematodes treated with kahweol and 16-*O*-methylcafestol, a derivative of cafestol. L1 stage nematodes were treated with either 1 µM kahweol, 1 µM 16-*O*-methylcafestol, or 0.1% dimethyl sulfoxide (vehicle control) for two days. The treatments did not affect the growth rate or viability of the nematodes. To identify cellular responses to low doses of these coffee diterpenes, RNA sequencing was performed on treated nematodes and compared to the control. Kahweol significantly regulated 107 genes, 84 of which were downregulated compared to the control. The differentially expressed genes were clustered into Gene Ontology (GO) terms. The most regulated pathways by kahweol include: the innate immune response, xenobiotic metabolic process, and oxidation-reduction process. Among the genes regulated by kahweol were *fat-5*, *ech-9*, and K05B2.4, which are related to fatty acid metabolic process. In contrast, 16-*O*-methylcafestol significantly regulated 56 genes, with 50 of them downregulated. There were no major differences in the pathways regulated by 16-*O*-methylcafestol compared to those affected by kahweol. Only two genes showed statistically significant differences between the treatments: *tsp-1*, involved in the innate immune response, and *oac-31*, predicted to encode acyltransferase activity. Overall, this study suggests that kahweol and 16-*O*-methylcafestol likely regulate similar molecular pathways at low doses. However, kahweol regulated more genes than 16-*O*-methylcafestol at the same dosage, suggesting that the differences on chemical structure change the biological activity of these coffee diterpenes.

478A Sequential multiplexed smFISH in *C. elegans* embryos

Sofia Foondos[1], Gabriela Sanchez[2], Thomas Underwood[2], Zachary Hemminger[2], Ryan Johnson[1], Roy Wollman[2], Pavak Shah[11]Molecular, Cellular, & Developmental Biology, UCLA, [2]Integrative Biology & Physiology, UCLA

Here we present an approach for sequentially multiplexed single molecule fluorescence in situ hybridization (smFISH) in worm embryos, aptly named "wrmFISH". While conventional smFISH approaches are limited by the number of separable fluorescent probes available (typically 4 or 5), wrmFISH enables the measurement of 10's of mRNA species in the same sample using a 2-step hybridization strategy where an encoding probe labels the target transcript and presents a unique barcode sequence that is then read out by sequentially hybridizing a fluorescent readout probe, imaging, chemically stripping the fluorophore from the previous readout probe, and repeating using automated fluidics and microscope control. We present a sample preparation protocol for optimized tissue clearing in C. elegans embryos and demonstrate wrmFISH by measuring transcript abundances of 15 cell cycle related genes that exhibit tissue and stage-specific variation in abundance and several of which show strong enrichment in the p-granules of the embryonic germline precursors. wrmFISH provides a novel approach for gene expression measurement in the worm embryo, providing a greater degree of multiplexing than conventional smFISH and HCR approaches while retaining many of their advantages.

479A Functional characterization of Argonaute syndromes variants in *Caenorhabditis elegans*

Belen Gaete Humada[1], Amelie Piton[2], Davor Lessel[3], Hans-Jurgen Kreienkamp[4], Victor Ambros[5], Anna Zinovyeva[11]Division of Biology, Kansas State University, [2]Department of Translational Medicine and Neurogenetics, Institute of Genetics and Molecular and Cellular Biology, Strasbourg University, [3]Institute of Human Genetics, University of Regensburg, [4]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, [5]Program of Molecular Medicine, UMass Chan Medical School

Precise regulation of gene expression is essential for animal development. Argonaute (AGO) proteins, guided by microRNAs (miRNAs), play key roles in post-transcriptional gene regulation by silencing target genes. Recently, *de novo* coding variants in the human AGO1 and AGO2 genes have been implicated in rare developmental disorders, collectively termed Argonaute syndromes (AS). The AS variants, most of which are missense, are associated with clinical manifestations that range from mild to severe. As the number of distinct AS variants continues to grow, there is a need to rapidly characterize the effects of these variants on the molecular functions of AGO proteins. Given that the affected amino acids are conserved between the miRNA-associated AGOs in humans and *Caenorhabditis elegans*, modeling *AGO1/2* mutations in *C. elegans* allows for rapid *in vivo* functional characterization of AS variants. Previously, we introduced four equivalent *AGO1* AS mutations in the *C. elegans* homolog *alg-1*, revealing unexpected and unique developmental and molecular phenotypes among the four modeled AS variants. Some *alg-1* AS mutations severely impacted miRNA function without impacting miRNA abundance while also producing unique effects on the mode of gene repression, highlighting the value of *C. elegans* models for functional analysis of AS variants. In this study, we expand our modeling efforts, further increasing our coverage of AS variations. Using CRISPR/Cas9 genome editing, we engineered ten additional *alg-1* AS variants for functional assessment. Genetic analysis of *alg-1* AS strains revealed varying degrees of developmental defects, consistent with previous findings. While some variants appear to cause only mild effects, a subset of *alg-1* AS mutations disrupt development more strongly than the reference *alg-1* null allele, exhibiting antimorphic phenotypes. Introducing wildtype *alg-1* into *alg-1* AS mutant strains partially restores normal development, consistent with dosage-dependent effects of ALG-1 function. Our current work is aimed at characterizing the molecular effects of *alg-1* AS variations in miRNA biogenesis and activity. Genetic and molecular characterization will enable functional classification of AS variants, contributing to our understanding of genotype-phenotype correlations for this disorder and paving the way for mechanistic studies connecting AGO dysfunction to AS pathogenesis.

480A RNA-binding protein HRPK-1 coordinates with miRNAs to regulate *C. elegans* development

Mika Ghosh, Li Li, Anna Zinovyeva Division of Biology, Kansas State University

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression post-transcriptionally. Argonaute proteins incorporate miRNAs and guide their binding to target mRNAs through partial sequence complementarity, leading to repression of target genes. In addition to miRNAs, RNA-binding proteins (RBPs) also regulate mRNA expression by influencing RNA processing, transport, and translation. RBPs are known to interact both genetically and biochemically with the miRNAs. Dysregulation of miRNAs or their interactors can alter gene regulation, contributing to diseases such as cancer. Aiming to identify regulators of miRNA function, we screened for protein interactors of the Argonaute protein ALG-1 in *Caenorhabditis elegans* via mass spectrometry. We found HRPK-1 to be a physical interactor of ALG-1. HRPK-1 is the homolog of human *hnRNPK,* a KH domain-containing RBP. Our previous work showed that HRPK-1 modulates miRNA activity during *C. elegans* development. Loss of *hrpk-1* enhances abnormal developmental phenotypes that are associated with reduced function of the *let-7* and *mir-35* miRNA families, both of which are key regulators of *C. elegans* development. To determine which HRPK-1 domains are essential for miRNA function, we performed a functional domain analysis by deleting or mutating six distinct HRPK-1 domains or predicted signaling sequences. We found that the RNA-binding activity of all KH domains is important for miRNA function and the proper development of *C. elegans.* Deletion or mutation of other HRPK-1 domains and signaling sequences had variable impacts on *C. elegans* development and miRNA activity. Since mutations in the human *hnRNPK* gene are linked with Au-Kline syndrome (AKS), a rare neurodevelopmental disorder, we wished to explore the link between AKS variants and miRNA-mediated regulation. To test the impact of AKS variants on HRPK-1 activity, we engineered the *hnRNPK* (L68R) mutation into the KH domain of *C. elegans hrpk-1*. Preliminary functional characterization of this mutant revealed that it enhances the developmental defects of *mir-48 mir-241(nDf51)* mutants, suggesting its involvement in miRNA-associated pathways. Overall, our research aims to elucidate how HRPK-1 coordinates with miRNAs to regulate *C. elegans* development, providing insights into the broader role of RBP coordination with miRNA-mediated gene regulation pathways.

481A NHR-49/PPARa Coordinates Nutritional Status, Oocyte Activation, and Resource Allocation to Balance Reproduction and Longevity in *C. elegans*

Sharada Gopal, Amaresh Chaturbedi, Siu Sylvia LeeMolecular Biology and Genetics, Cornell University

Coupling nutritional availability to fertility is essential for embryonic survival and the management of parental resources. The nuclear hormone receptor NHR-49, a homolog of PPARα, is a key transcriptional regulator of nutrition sensing and fatty acid metabolism in *Caenorhabditis elegans*. Here we uncover a crucial role of NHR-49 in reproduction - controlling oocyte activation and maturation. Loss of NHR-49 leads to inappropriate oocyte activation and ovulation in the absence of sperm. This phenotype occurs through a mechanism independent of the germline sperm-sensing VAB-1 pathway and primarily involves NHR-49's action in somatic gonadal sheath cells rather than in the germline itself. Using feminized mutants that lack functional sperm, we further demonstrate that inappropriate oocyte activation is accompanied by premature depletion of lipid and shortened lifespan. This is due to the misallocation of the lipoprotein complex yolk into the unfertilized oocytes, contributing to the shortened lifespan of mutant worms lacking *nhr-49*. In a more physiological context, we show that NHR-49 couples nutritional status to germline proliferation, as evidenced by its requirement for the starvation-induced reduction in germline proliferation. These findings establish NHR-49 as a critical coordinator of nutrition sensing, reproduction and metabolism, ensuring appropriate resource allocation between soma and germline, and balancing reproductive success and longevity.

482A Investigating the role of *prg-1* and *rde-3* in small RNA mediated male fertility and epigenetic inheritance in *C. elegans*

Katrin Gross[1], Colin Conine[21]Children's Hospital of Philadelphia, [2]University of Pennsylvania School of Medicine

Endogenously expressed small RNA pathways regulate a diverse array of physiology in *C. elegans* and are particularly important for reproduction. Canonically, this regulation is initiated through recognition of a target mRNA by an Argonaute protein loaded with a primary small RNA. This triggers the production of secondary small RNAs (called 22G-RNAs) that amplify the regulatory response. 22G-RNAs are also responsible for the transmission of epigenetic information across generations. However, the triggers initiating the biogenesis of 22G-RNAs and their subsequent accumulation in mature gametes remain poorly understood. In this study, I investigate key factors in the biogenesis of silencing 22G-RNAs: PRG-1, a PIWI Argonaute that binds primary-acting 21U-RNAs(piRNAs), and RDE-3, a ribonucleotidyltransferase involved in the recruitment of RNA dependent RNA polymerase. *prg-1* and *rde-3* are expressed in the germline and mutants of both exhibit a mild reduction in fertility when newly homozygous (P0). However, *prg-1* mutants become sterile (mortal germline phenotype - *mrt*) after being homozygous for 10+ generations. Interestingly, we find that *prg-1* males and females retain a mild infertility phenotype to at least generation 10, at which point they incur a stochastic (can occur anywhere from generation 10 to 30) catastrophic loss of fertility. This sterility is associated with reduced function of both sperm and oocytes. Strikingly, we find that loss of *rde-3* suppresses the *mrt* phenotype of *prg-1* mutants, as *prg-1 rde-3* mutant males and females retain fertility well beyond 30 generations. To identify the small RNAs and mRNAs that are regulated by *prg-1* and *rde-3*, I performed small RNA and mRNA-seq from generations 0 to 20 of *prg-1, rde-3*, and *prg-1 rde-3* mutants, focusing on the male germline. While previous studies have proposed that RDE-3 functions downstream of PRG-1 in a linear 22G-RNA-producing pathway, my findings additionally identify 22G-RNAs that are RDE-3 dependent and PRG-1 independent, suggesting a novel pathway downstream of RDE-3 also occurs in the male germline. Importantly, this pathway may be responsible for the *mrt* phenotype in *prg-1* mutants, as it leads to the mis-regulation of germline genes, potentially as a consequence of germline mis-expression of somatic genes. These findings reveal a novel layer to the complexity of small RNA-mediated regulation of germline gene expression and its transgenerational effects on fertility.

483A The phosphatase PIR-2 interacts with multiple proteins to regulate germline development

Swati Srivastava[1], Adrian Toledo[1], David Gorrie[1], Lars Luscher[1], Ahmad Adrian[1], Ayaan Ismail[1], Rohit Niradi[1], Weifeng Gu[21]UC Riverside, [2]Univ of Calif Riverside

The PIR-1 and PIR-2 proteins belong to a conserved superfamily of phosphatases, whose members modifies proteins, RNAs or both. Unlike most other phosphatases, many members of this family, are localized in nuclei, suggesting they are involved in regulating gene expression or DNA replication. Since this family has been understudied likely due to lack of phenotypes in cell culture systems and molecular cues, the functions of these proteins are mostly unknown. We previously demonstrated PIR-1 is an RNA polyphosphatase removing the last two phosphates from triphosphorylated RNAs, and PIR-1 is required for the biogenesis of 26G-RNAs, which regulate ~1,500 male-germline specific genes including those for MSPs and SSPs. Like PIR-1, the paralog PIR-2 alone is also required for fertility, suggesting PIR-1/PIR-2 each play some non-overlapping roles. We used CRISPR to tag the endogenous PIR-2 with Degron for conditional knockdown, TurboID for proximity labeling, and GFP for monitoring localization. We found PIR-2 is primarily localized in all nuclei and displays higher expression levels, especially in somatic cells. PIR-2 depletion generates smaller and immature germlines with few oocytes. We found PIR-2 may affect small RNA biogenesis based on the profile change of small RNAs in the PIR-2 depletion worms. We used proximity labeling to identify several PIR-2 interacting proteins including PGL-1, proteins involved in RNA splicing and others. Consistently, we found depletion of PIR-2 generated abnormal and bigger PGL-1 aggregates around the nuclear envelope, and these P granule loci become less stable and quickly diffuse to cytoplasm when living samples were monitored under a microscope, suggesting that PIR-2 may be involved in regulating P granule assembly and/or stability. This is consistent with the observation of the small RNA profile changes since P granules play important roles in small RNA biogenesis, stability and functions. We are also investigating if and how PIR-2 regulates RNA splicing and how the PIR-2 interaction affects the expression and localization of other PIR-2 interacting proteins. To understand the molecular functions, we are examining if PIR-2 is an RNA or protein phosphatase using in vitro assays and if PIR-2 affects protein modifications on PGL-1. Our study constitutes the first systematic investigation on a member of this conserved nuclear phosphatase family with clear molecular cues and functions.

484A Expanding the genetic toolkit of *C. elegans*: Efficient gene activation with sgRNA feeding-based CRISPRa

Rohil Hameed, Alaattin Kaya Biochemistry and Molecular Genetics, University of Virginia

The nematode *Caenorhabditis elegans* is a pivotal model organism in genetics, developmental biology, and neurobiology due to its simplicity, transparency, and well-characterized genome. Since its genome was sequenced in 1998, *C. elegans* has provided fundamental insights into conserved biological processes. Traditional genome manipulation techniques, such as EMS mutagenesis and RNA interference (RNAi), have been complemented by more precise tools, including CRISPR/Cas9 and transposon-based insertional mutagenesis, enabling refined gene knock-out and knock-down studies.

Among these, Mos1-mediated Single-Copy Insertion (MosSCI) remains a widely used approach for stable transgene expression. However, its labor-intensive and costly nature limits its application in high-throughput genetic screens. To address this challenge, we introduce a novel single-guide RNA (sgRNA) feeding-based methodology leveraging CRISPR activation (CRISPRa). This approach utilizes bacteria-expressing sgRNAs to enhance gene expression efficiently and systematically.

We demonstrate the utility of this method through pilot studies in two novel *C. elegans* models: one investigating gene overexpression effects on aging and the human alpha-synucleinopathy disease model. Our findings highlight the advantages of sgRNA feeding-based CRISPRa for high-throughput screening, allowing systematic activation of one or more genes with minimal technical constraints, indicating its flexibility for high-throughput screening.

Furthermore, integrating MosSCI with sgRNA feeding-based CRISPRa significantly expands the genetic toolkit available for *C. elegans* research. This combined approach provides both precise and stable transgene expression alongside efficient gene activation, reinforcing *C. elegans* as a versatile and powerful model for functional genomics and gain of function studies.

Key Words: Gene Regulation and Genomics, *C. elegans,* Gene Overexpression, sgRNA toolkit, Aging and Stress.

485A KH-domain protein FUBL-1: a new player in the ERGO-1 small RNA pathway?

Andrea Hinas[1,21]Stockholm University, [2]Uppsala University

RNA interference (RNAi) is induced by small RNAs that are produced endogenously (endo-RNAi) or from exogenous double-stranded (ds)RNA (exo-RNAi). The small RNAs act by guiding Argonaute (AGO) proteins to target mRNAs to down-regulate gene expression. RNAi can also be inherited for one or more generations to affect gene expression in progeny.

Here, we add a piece to the small RNA puzzle by initial characterization of the role of the putative RNA-binding protein FUBL-1 in small RNA silencing in *C. elegans*. FUBL-1 belongs to a conserved family of KH-domain proteins with functions in transcriptional regulation, splicing and RNA stability. In *C. elegans*, *fubl-1* was previously identified in a screen from the Ruvkun lab, where *fubl-1* knockdown was shown to result in reduced GFP RNAi efficiency. We confirmed this observation but also found that genetic loss of *fubl-1* results in the opposite phenotype, i.e. enhanced exo-RNAi. We further show that the enhanced RNAi effect is primarily due to a stronger progeny response to inherited RNAi. Both nuclear and cytoplasmic RNAi is enhanced, indicating that *fubl-1* acts upstream of small RNA loading into nuclear and cytoplasmic secondary AGOs, respectively.

Mutants with enhanced exo-RNAi are often defective in endo-RNAi mediated by the AGO ERGO-1. In line with this, RT-qPCR of ERGO-1 target mRNAs showed upregulation in *fubl-1* mutant animals, supporting a role for *fubl-1* in silencing of these genes. Taken together, our study introduces FUBL-1 as a new player in the ERGO-1 pathway and inherited RNAi. Moreover, the opposite phenotypes observed upon RNAi-mediated knock down and genetic loss of *fubl-1*, respectively, further stress the importance of detailed functional studies to complement RNAi screens.

486A Glia and other cell types exhibit tissue-specific oscillatory gene expression during development

Alexis Weinreb[1], Rachel Swope[2], JB Burnett[2], Wendy Fung[2], Manasa Basavaraju[1], Marc Hammarlund[1], Maxwell Heiman[21]Yale School of Medicine, [2]Harvard Medical School, Boston Children's Hospital

Many animals exhibit oscillatory physiological cycles on the scale of hours (circadian cycle) to weeks (estrous cycle). These cycles are coordinated across tissues, but it remains unclear if they involve primarily tissue-specific changes or a shared global program. Here, we use single-cell transcriptional profiling of *C. elegans* to extract both tissue-specific and shared patterns of oscillatory gene expression across cell types. Previous whole-animal transcriptomic analyses of tightly-synchronized larval populations identified thousands of genes with oscillatory expression, many of which are associated with molting and are broadly expressed in hypodermis and seam cells (*1–4*). By contrast, we recently identified apical extracellular matrix (aECM) proteins that are expressed exclusively during molting in a single glial subtype (the six ILso glial cells), suggesting that some oscillatory genes have highly restricted expression. To examine and compare oscillatory gene expression in individual cell types, we used FACS to generate samples enriched for ILso glia, but also containing all major tissue types, and analyzed them by single-cell RNA-Seq. Our data confirm the presence of oscillatory gene expression in specific cell types (e.g. hypodermis, pharynx, socket glia) and demonstrate that gene expression in other cell types is not oscillatory (e.g. neurons, muscles, sheath glia). By temporally ordering single cells to model oscillatory gene expression as a function of pseudotime, we identified genes displaying cyclic behavior in each individual cell type. We observed gene families specifically oscillating in particular tissues, and expanded the number of known oscillating genes twofold, presumably owing to our ability to capture patterns restricted to rare cell types. Our results reveal how organism-wide oscillations coordinate complex patterns of cell type-specific gene expression and provide insights into the regulatory networks at play within individual cell types.

1. Kim *et al.*, *Nat Genet*. 45, 1337–1344 (2013); 2. Hendriks *et al.*, *Mol Cell*. 53, 380–392 (2014); 3. Meeuse *et al.*, *Mol Sys Biol*. 16, e9498 (2020); 4. Meeuse *et al.*, *The EMBO Journal*. 42, e111895 (2023)

487A Collagen Gene Family Evolution in *Caenorhabditis elegans*

Raymond Hendricks[1], Mary Ladage[1], Rajeev Azad[2], Einav Gross[3], Pamela Padilla[21]Biology, University of North Texas, [2]University of North Texas, [3]Hebrew University of Jerusalem

Collagen proteins are found in the extracellular matrix of a wide range of metazoans, but in some species, such as *C. elegans*, this gene family has undergone significant expansion. There are 180 known or predicted protein coding collagen genes in the *C. elegans* genome, whereas there are only 44 collagen genes in the *Homo sapiens* genome. Multiple sequence alignment indicates 80% protein sequence similarity among the collagen proteins encoded from 46 collagen genes in *C. elegans*. Gene family expansion is often associated with adaptive divergence. However, an in-depth analysis of collagen gene evolution in *C. elegans* has not been conducted. To investigate the collagen gene family expansion, an evolutionary reconstruction of the collagen gene family was completed using genome and proteome sequence data from sixteen species; four of which are from the *Caenorhabditis* genus and twelve are from other representative metazoan species (five vertebrates, seven invertebrates). To set the foundation of the evolutionary distance between these species and the number of gene duplications within each species, we used OrthoFinder to analyze the complete proteomes. From these identified orthogroups, the C. elegans collagen evolution was further defined and analyzed using the MEGA software and ape and phangorn R packages. Furthermore, a maximum likelihood phylogenetic tree was constructed using the collagen gene sequences alignments model and the best fit model was used. The sequence homology level trees, produced for DNA or protein sequences, were compared using the Robinson-Foulds distance method. A Time tree analysis was completed with MEGA to estimate evolutionary divergence among species. The findings from these evolutionary studies will be presented. Thus far, our findings suggest distinct yet overlapping functions, redundancies, and inferred operon control of collagen genes.

488B Argonaute Space-Time: Characterizing the regulatory mechanisms of Argonaute proteins in small RNA pathways during germline development in *Caenorhabditis elegans*

Ismail Irshaid, Julie Claycomb Molecular Genetics, University of Toronto

Small RNA pathways are conserved gene regulatory systems found across all domains of life. In these regulatory pathways a small RNA cofactor provides sequence specificity to Argonaute proteins, the central effectors. Together, the small RNA cofactor and Argonaute proteins comprise the RNA-induced Silencing Complex, which can exert its influence on all stages of a transcript's life cycle, from transcription to translation.

The nematode *Caenorhabditis elegans* has proven to be a champion of small RNA biology, featuring an expanded small RNA pathway of 19 Argonautes and 4 endogenous small RNA types. In spite of this rich foundation for understanding small RNA pathways, a major missing piece of the puzzle regards the mechanisms by which Argonautes proteins regulate their targets. In *Caenorhabditis elegans*, majority of Argonautes appear incapable of endonucleolytic cleavage, in that they lack the conserved catalytic tetrad responsible, and are presumed to exert their regulatory effects by recruiting other enzymatic co-factors and effector complexes to elicit alternative regulatory outcomes. However, by which specific molecular mechanisms and regulatory levels remains unknown. Conversely, for the majority of Argonautes possessing (putative) conserved catalytic tetrad, the functionality and role of this feature in Argonaute/small RNA-mediated regulatory mechanisms *in vivo* remains open ended, as they have not been directly studied.

To this end, I am systematically characterizing the regulatory mechanisms of both catalytic and non-catalytic Argonautes in *Caenorhabditis elegans*. I will identify Argonautes that act at the transcriptional and post-transcriptional level by profiling the transcriptome of an array of single *argonaute* mutants and wild-type worms by RNA-sequencing. To identify Argonautes that act at the translational level, I will employ a two-pronged approach, examining the association of target transcripts with ribosomes via Translating Ribosome Affinity Purification sequencing and target protein levels via mass spectrometry-based proteomics in single *argonaute* mutants versus wild-type. By integrating transcriptomic, translatomic, and proteomic analyses, I will uncover target-specific and Argonaute-specific regulatory mechanisms, providing an unprecedented, high-resolution portrait of Argonaute regulatory mechanisms in this important model system.

489B A parallel array of RNA molecules spans nuage subdomains during inherited RNA silencing

Takao Ishidate, Daniel Durning, Aaron Wheeler, Wendy Tan, Craig Mello RNA Therapeutics Institute, UMass Chan Medical School

In animal germlines, peri-nuclear, membrane-less organelles (nuage) are comprised of subdomains enriched with factors that regulate transgenerational gene silencing. For example in C. elegans, nuage contains mutator foci enriched for factors, including the poly-UG polymerase, RDE-3, and other factors involved in amplifying small RNA signals, a UPF1 helicase ZNFX-1 resides in an adjacent Z granule, and Argonautes are enriched in yet other granules distal to the Z granule. However, how these subdomains interact with target RNA during transgenerational silencing remains poorly understood.

Using fluorescent in-situ hybridization (smiFISH), we examined target RNA localization in L1 larvae undergoing inherited RNAi. We found that RNAi-exposed L1 larvae exhibit a dramatic accumulation of target RNA in an enlarged domain of nuage. Remarkably, RNA within this enlarged domain form a parallel, polarized array coinciding with distinct nuage protein zones. The 3' dsRNA-targeted region localizes within a zone enriched for poly-UG signals, while the 5' non-targeted region resides in a distinct nuage subdomain. Additionally, the accumulated RNA lacks a polyA tail but instead carries a poly-UG tail, likely added by RDE-3.

Our findings suggest that inherited RNAi induces a unique accumulation within nuage of a parallel array of target RNA. This spatial organization may focus silencing amplification within the targeted region while preventing uncontrolled spread along the RNA. Thus, the previously described formation of subdomains within nuage could reflect coordinated, compartmentalization of events along target RNA molecules. The formation of these organized structures implies mechanisms that anchor, bundle and straighten RNA within nuage. Ongoing experiments aim to further elucidate these interactions and their role in transgenerational gene silencing.

490B Development of a heat-inducible gene expression system in *Pristionchus pacificus*

Yuuki Ishita[1], Takahiro Chihara[2], Misako Okumura[21]Brain Research Institute, Niigata University, [2]Graduate School of Integrated Sciences for Life, Hiroshima University

The precise control of gene expression in a temporal manner is crucial for understanding dynamic biological processes. In *Caenorhabditis elegans*, the heat-shock promoter has been widely used to induce gene expression upon heat shock. *Pristionchus pacificus* has been established as a satellite model nematode of evolutionary biology. In *P. pacificus*, genetic tools such as an annotated genome, CRISPR/Cas9 genome editing, and gene expression via complex arrays are available; however, a temporal gene expression system has not yet been applied. To establish a temporal gene expression system using a heat shock promoter, we investigated endogenous heat-shock promoters in *P. pacificus*. RNA-seq analysis revealed that the orthologs of *hsp-16.41* were upregulated upon heat-shock treatment. We generated the reporter transgenic animals for these genes and found that the promoter of one of the *hsp-16.41* orthologs could induce gene expression upon heat-shock events. This gene was highly induced throughout the larval stages. We also examined the effects of heat shock during each larval stage on development, predatory behavior, and mouth-form plasticity. Heat shock slightly delayed the development and affected the mouth form plasticity. In summary, we identified a potential heat-shock promoter that could be applied to the temporal gene expression system in *P. pacificus*. Furthermore, we would like to discuss the application of a FLP/FRT system optimized for *P. pacificus* in combination with the heat-inducible gene expression system.

491B Modular protein tags using PhIT

Adam J Hefel, Kevin J Kruse, Matthew S Rich, Kaden R Wall, Erik M JorgensenSchool of Biological Sciences, University of Utah

Tagging proteins to determine their localization allows researchers to infer protein function. Currently proteins are tagged at endogenous loci using CRISPR. However, CRISPR insertions are error-prone. Large (>1kb) insertions can be difficult to insert and verify with PCR, and are generated at lower frequencies. Furthermore, CRISPR tagging requires a different set of reagents for every locus and every desired tag. By contrast, recombinases can insert DNA sequences in an error-free and modular manner.

Here, we use the integrase PhiC31 to tag endogenously expressed proteins. PhiC31-mediated insertion of tags ('PhIT') takes advantage of the small PhiC31 target site (attB, 39bp) which is inserted at either the N- or C-terminus of the target protein. These small landing pads are easy to generate with CRISPR and easy to verify with PCR and sequencing. The PhiC31 recombinase integrates the desired tag from plasmid reagents into these attB landing pads without error. Current PhIT tags include fluorescent proteins, degrons, or superresolution tags. These tagging plasmids can be used at different attB landing pads, and each attB is a substrate for a variety of tags, providing a modular approach to tagging a single gene.

To eliminate the insertion of plasmid backbone sequences, a second recombinase is used to reduce the plasmid to only the tag and the complementary attP recombination site which recombines with the attB landing pad. Since the tyrosine recombinases Cre and FLP are usually dedicated to other applications, two tyrosine recombinases (B3 and Dre) were developed for use in the *C. elegans* germline. Injected plasmid DNA of the tag and the recombinases form extrachromosomal arrays briefly, but these are resolved into small DNA circles by the tyrosine recombinase to the minimal tag sequence. PhiC31 integrates the tag into the attB landing pads perfectly with only the small scars from the recombinase target sites.

Because protein tag plasmids can be injected and maintained as arrays when recombinases are not present, crossing of tag arrays can be used as a method to generate PhIT tags. By crossing the strains expressing the recombinases, the attB target site, and the array with the fluorescent tag of choice, a new tag is generated. Thus, a collection of strains with attB sites in different genes can be crossed to a library of arrays with different tags to provide modular tags at any locus. This makes gene tagging broadly available to any lab or classroom.

492B Determining the mechanism of FUdR-mediated inhibition of the mitochondrial unfolded protein response

Nathaniel Jordan[1], Suzanne Angeli[21]Molecular and Biomedical Sciences, University of Maine, [2]University of Maine

The mitochondrial unfolded protein response (mitoUPR) normally is activated in order to target misfolded proteins in the mitochondrial matrix and maintain mitochondrial protein homeostasis. We have identified that 5-fluoro-2'-deoxyuridine (FUdR), a DNA synthesis inhibitor, potently inhibits the mitoUPR in C. elegans as measured by the mitoUPR GFP reporter . However, the mechanism of how FUdR inhibits the mitoUPR is unknown. Previous studies have demonstrated that FUdR can activate the heat shock response (HSR) independent of heat stress (Angeli et al, 2013; Brunquellet al, 2014). To determine whether FUdR may inhibit the mitoUPR by activating the HSR, we will examine a strain with a mutation in hsf-1, the main transcription factor associated with the HSR. We will cross the mitoUPR reporter strain (phsp-6::GFP) with the hsf-1(sy441) mutant and determine whether they are capable of inducing a robust mitoUPR in either the absence or presence of FUdR. To induce the mitoUPR, we will utilize RNAi to knock down the F-ATP synthase subunit OSCP/atp-3 (Angeli et al., 2021)Results from this research will give us insights as to the mechanism of FUdR-mediate inhibition of the mitoUPR.

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493B InVivo Biosystems: Lessons & Highlights from 16 Years of Transgenic Techniques Development and Troubleshooting

Jennifer A. Lawson, Ben Jussila, Kalyn M. Hubbard, Chesley Morgan Johnson, Joseph James Bruckner, Trisha J. Brock InVivo Biosystems

InVivo Biosystems serves the *C. elegans* community by building bespoke transgenics strains for research. To date our team has created and delivered over 4,200 custom *C. elegans* projects to academic and industrial clients worldwide. Our genome editing work encompasses a plethora of genomic edits and editing contexts, including precise knock-in of patient mutations, whole gene replacements, knockouts, conditional alleles, and many varied background mutant strains, and non-*C. elegans* nematode species. Here, we highlight a published customer story in collaboration with Dr. Rachel Arey, spanning the project design, early rescue and overexpression transgenic strain generation, to the creation of custom humanized strains using our Whole-gene Humanized Animal Model (WHAM) platform. Our broad genome editing expertise and robust transgenesis pipeline enabled our team to design and deliver 5 highly customized transgenic strains in less than 8 months. These strains facilitated an elegant series of behavioral and biochemical experiments in the Arey laboratory ultimately resulting in *in vivo* functional validation of pathogenic patient variant contributions to learning and memory deficits in *C. elegans*. This body of work culminated in a recent publication (Hayden et al., 2024). These examples and the other genome editing vignettes presented here highlight the value of the highly collaborative and customized services we offer the community as a contract research organization.

494B Exploring Enzymatic and Proteostatic Genes in Exopher Production

Mark Abbott, Ilija Melentijevic, Ryan Guasp, Caroline Kaprelian, Sarah Farah, Monica Driscoll Genetics, Rutgers

Proteostasis is crucial for cellular function and can occur both inside and outside cells. Large vesicle extrusions in C. elegans that were named exophers may be responsible for the transfer of protein aggregates to neighboring cells. The exact genetic mechanisms involved in exophergenesis are largely unknown. To investigate exopher production, a whole genome RNAi screening was conducted, with RNAi knockdown targeting a single gene. Images were captured using a high-content imager, revealing varying rates of exopher production across different genes. This project focuses on reassessing 91 genes with low exophergenesis rates in the ZB5786 strain with wildtype sem-2. RNAi bacterial clones were grown and fed to C. elegans. Once images were taken, exopher counts were recorded. By testing these 91 gene clones in the ZB5786 strain and comparing them to our whole genome screen in ZB4559, this research aims to confirm candidate hits and the genetic factors driving exophergenesis.

495B *In-vivo* tethering H3K9 methyltransferase induces silencing in *cis* and the *de-novo* production of trans-acting small RNAs

Sara A Keane, Craig C Mello, Masaki Shirayama RNA Therapeutics Institute, UMass Chan Medical School

Histone modifications regulate gene expression by altering chromatin structure and DNA accessibility. In *C. elegans*, transgenerational gene silencing involves the interplay between small RNA/Argonaute pathways and chromatin modifications, however, whether and how heterochromatin can induce small RNA production remains unclear.

To investigate the influence of histone modifications on gene expression, we developed an assay system that allows targeted recruitment of a specific histone modifier to a gene locus. Our reporter consists of a single-copy *cdk-1* gene fused to *gfp*, with eight Lac operator (*lacO*) sequences positioned upstream of the *cdk-1* promoter. These *lacO* sites serve as binding sites for the Lac repressor (LacI).

H3K9 methylation (H3K9me), catalyzed by MET-2 and SET-25, is a repressive epigenetic marker associated with gene silencing and heterochromatin formation. As expected, tethering LacI::MET-2 or LacI::SET-25 to the *lacO::cdk-1::gfp* reporter led to complete silencing in both somatic and germline cells. Interestingly, tethering also caused transitive silencing of a second *gfp* reporter (lacking *lacO* sites) located on another chromosome. Knocking out *rde-3*, a gene encoding a poly(UG) polymerase essential for WAGO-22G small RNA synthesis, prevented transitive silencing without restoring expression of the *lacO::cdk-1::gfp* reporter itself. As expected, small RNA sequencing confirmed that tethering induces WAGO-22Gs targeting the *gfp* sequences.

These findings suggest that H3K9me deposition can initiate both *cis*-silencing by heterochromatin formation and *trans*-silencing via *de novo* WAGO-22G production. We are currently using this reporter assay to identify genetic factors required for both types of silencing and to characterize transcripts produced at the reporter locus.

Histone modifications have been shown to alter multiple steps in gene expression including transcription, RNA splicing, stability, nuclear export, and translation efficiency. Deciphering chromatin signals is complex, as multiple modifications can occur simultaneously. Our *lacO*-LacI system should help us dissect these combinatorial effects and allow us to temporally order the silencing events required for *cis*- and *trans* silencing.

496B Regulation of Argonaute activity by an N-terminal IDR

Rene F Ketting[1], Ida Isolehto[1], Lizaveta Pshanichnaya[1], Svenja Hellmann[1], Diego Páez Moscoso[1], Kumar Gaurav[2], Lukas S Stelzl[1,2], Jan Schreier[1,3], Jiaxuan Chen[1], Fridolin Kielisch[11]Institute of Molecular Biology, [2]Faculty of Biology, Johannes Gutenberg University, [3]EMBL Monterotondo (Current)

Argonaute proteins are key key players in RNA interference (RNAi) pathways. By binding small RNA co-factors, they can target transcripts in a sequence-specific manner and silence them. However, how Argonautes are controlled themselves is not well understood. In *C. elegans*, a major class of Argonaute proteins is the WAGO clade. WAGO's aquire their small RNA co-factors (22G RNAs) from RNA dependent RNA polymerase activity and act in RNAi pathways that silence a wide variety of targets. Many WAGO's, and in fact many Argonaute proteins in general, contain an N-terminal domain that is intrinsically disordered (IDR). Their functions are unknown. We are studying the N-IDR of WAGO-3, an Argonaute acting in paternal inheritance of RNAi, to understand the potential regulatory role(s) of this poorly studied domain.

We found that deletion of the WAGO-3 N-IDR results in sterility. WAGO-3(DIDR) erroneously binds 22G RNAs that target histone transcripts. This is accompanied by severe developmental defects of the gonad, most notably a strong under-proliferation during larval development. Binding of the 22G RNAs is required for this effect and mutation of histone turnover machinery can partially rescue the effect. We also found that WAGO-3(DIDR) acts through the nuclear WAGO protein HRDE-1, just like it does in RNAi inheritance. How the IDR controls the loading of WAGO-3 is not fully clarified yet, but our experiments indicate that the IDR may regulate WAGO-3 loading by physically binding within the RNA-binding cleft of this Argonaute. Deletion of the WAGO-1 N-IDR also results in sterility. Whether this also involves erroneous 22G RNA binding is being tested.

In parallel, we study how two proteases, DPF-3 and APP-1, jointly process the WAGO-3 N-IDR. DPF-3 has previously been shown to process WAGO-3 and to contribute to its stability. We show biochemically that DPF-3 and APP-1 jointly process the N-IDR fully, and both *dpf-3* and *app-1* mutants are RNAi inheritance defective, just like *wago-3* mutants. Interestingly, DPF-3's stabilizing effect on WAGO-3 does not depend on processing, as DPF-3-resistant *wago-3* mutants still require DPF-3 for stability. Interestingly, such *wago-3* mutants are defective in RNAi inheritance.

Based on the two studies combined, we will present a model explaining the role the N-IDR and its processing in WAGO-3 function. This works sets an important paradigm for how auto-regulation of Argonaute function may be driven by their IDR domains.

497B Temporal gene expression shifts underlying transcriptomic divergence of developmental trajectories in *Caenorhabditis* species

Rupa Khanal, Christopher Large, Junhyong Kim, John I Murray Biology, University of Pennsylvania

Precise timing of gene expression is critical for developmental processes, yet how evolutionary changes in expression timing contribute to developmental divergence remains poorly understood. While developmental programs and cell divisions are often conserved between closely related species, subtle temporal changes in gene expression might accumulate and lead to transcriptomic divergence, although the extent and functional significance of these temporal shifts are not fully known. The highly conserved embryonic lineages of Caenorhabditis elegans and C. briggsae provide an ideal system to investigate these temporal shifts at single-cell resolution. In this study, we quantified temporal gene expression divergence between C. elegans and C. briggsae embryos using single-cell RNA sequencing (scRNA-seq) data, defining homologous developmental trajectories by tracing gene expression dynamics across annotated progenitor lineages from the 28-cell stage to terminal differentiation.

To quantify temporal divergence, we used Dynamic Time Warping (DTW) to align gene expression profiles across lineage-defined trajectories and identified significant temporal shifts for 5,485 genes in at least one of 123 trajectories. About two-thirds of these genes exhibited differences in persistence of expression (e.g., prolonged presence of certain maternal transcripts in C. briggsae), while the remaining third displayed shifts in onset timing, such as delayed onset of some cilia-related genes in C. elegans. These temporal shifts explain a modest but statistically significant portion of the overall gene expression divergence between the species.

To further dissect the regulatory basis of these temporal shifts, we are analyzing the temporal ordering of gene activation and deactivation events within individual lineage trajectories. We will investigate how temporal expression patterns of genes, including transcription factors (TFs) and regulators, vary across lineages and tissues within species, and relate these variations to changes in TF binding. Further integrating with chromatin accessibility data will help define temporal control mechanisms. Our study establishes a framework to identify temporally regulated genes in development and investigate their regulatory mechanisms. Keywords: *C. elegans, C. briggsae*, single-cell RNA-seq, dynamic time warping, Jensen–Shannon divergence, temporal regulation, development, evolution

498B Beyond model species: Single individual wild-caught nematode genomes and their insights

Erna King[1], Lewis Stevens[2], Witek Morek[2], Oleksandr Holovachov[3], Christopher Laumer[4], Mark Blaxter[21]Tree of Life, Wellcome Sanger Institute, Wellcome Sanger Institute, [2]Tree of Life, Wellcome Sanger Institute, [3]Swedish Museum of Natural History, [4]Natural History Museum

Nematode genomics has gone from strength to strength since the worm *C.elegans* was first sequenced, but the vast majority of the diversity of this ubiquitous phylum are unculturable and too small and heterozygous for traditional genome sequencing approaches. Capturing the diversity of these missing groups, particularly the predominantly small and free-living basal class Enoplea and central Chromadorea, is necessary to further our understanding of the evolutionary history of Nematoda. We have applied the Picogram Input Multimodal Sequencing (PiMmS) technique to sequence high quality genomes and transcriptomes of single specimens of 35 free-living nematode species of under-represented orders across the phylum. We combined our genomes with publicly available genome and transcriptome assemblies to build a comprehensive nematode phylogeny that supported the established tree but recovered the Enoplean class Ironida as polyphyletic, with family Leptosomatidae the sister group to the order Enoplida and Oxystominidae to Oncholaimida. The Chromadorean orders Leptolaimida, Plectida and Desmodorida were also polyphyletic and Monhysterida paraphyletic, highlighting the need for revision of these orders. For four of these wild-caught, free-living species, we also generated chromatin conformation capture (Hi-C) data, which allowed us to scaffold those assemblies into complete chromosomes. We found that the ancestral rhabditid linkage groups - the Nigon elements - can be traced deep into central Chromadorea, but not across the divide with Enoplea. Further wild-caught nematode genomes are being sequenced within the 959 Nematode genome project to elucidate the ancestral linkage groups for basal Nematoda and lend evolutionary context to the genomes of model nematode species.

499B Exploring the regulation and function of an ancient microRNA family in *C. elegans*

Kasuen Kotagama[1], Rima Sakhawala[1,2], Acadia Grimme[1,2], Katherine McJunkin[31]National Institute of Diabetes and Digestive and Kidney Diseases, [2]Johns Hopkins University, [3]Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally by targeting mRNAs for translational inhibition and degradation. MiRNAs are functionally categorized into families based on nucleotides 2-7 at their 5' ends, known as the "seed" region. Members of a miRNA family share the same seed sequence and are often functionally redundant due to their largely overlapping targets. A single miRNA can regulate many target transcripts, and identifying functional miRNA targets can be difficult. The *mir-51/100* family of miRNAs is widely conserved across metazoans, and its precise expression and regulation is essential for embryonic development in *C. elegans*. In the worm, this miRNA family consists of six mature miRNAs, *mir-51* through *mir-56*, and loss of the entire *mir-51* family is embryonically lethal with defects in elongation and organogenesis. The *mir-51* family members are functionally redundant when rescuing embryonic lethality indicating that the *mir-51* family probably shares a core set of essential targets; however, the essential target genes regulated by the *mir-51* family remains unknown. Hypomorphic *mir-51* family mutants, with deletions in some of the *mir-51* family members (*mir-52, mir-54-56*), bypass embryonic lethality. These hypomorphic mutants display post-embryonic phenotypes, which include developmental delays, reduced brood size, mating defects and food avoidance. We have performed tissue-specific rescues of the *mir-51* family in a hypomorphic background and found that the *mir-51* family acts in the pharynx and neurons to regulate development. To identify the genes targeted by the *mir-51* family, we performed a targeted RNAi screen, mRNA-sequencing, and tissue-specific rescue experiments. Based on these experiments, we identified a set of 27 putative *mir-51* family targets and validated the most promising candidate F26A3.4 as a molecular target of the *mir-51* family. We mutated the miRNA binding sites in all 27 putative target genes using a multiplexed CRISPR approach and generated multiple mutant strains, some with up to eleven mutated miRNA binding sites. These multiple binding site mutants replicate subsets of the gene expression changes observed in *mir-51* family hypomorphs, but do not recapitulate their deleterious phenotypes. This study suggests that deleterious loss of function phenotypes of the *mir-51* family may occur through the synthetic interactions among multiple upregulated target genes.

500B Drugging Worms: Examining the Molecular and Behavioral Effects of Acute Cocaine Exposure

Shelby Nyree Lauzon, Prince Mosley, HaoSheng Sun CDIB, The University of Alabama at Birmingham

The molecular landscapes of post-mitotic neurons are highly responsive to environmental stimuli, leading to dynamic changes in gene expression and cell function, affecting organismal behavior. Cocaine, a potent stimulant, increases the levels of the neurotransmitters dopamine and serotonin, resulting in neuronal over activation. To elucidate the transcriptional and chromatin-based mechanisms underlying cocaine exposure, we utilize Caenorhabditis elegans as a model organism, leveraging its compact, well-delineated nervous system and conserved neurobiological systems underlying drug responses. *C. elegans* offers unique advantages, including whole-nervous-system analysis at single-neuron resolution and in vivo high-throughput screening capabilities. We tracked the locomotion of adult *C. elegans* across varying cocaine concentrations to establish a dose-response curve. Concurrently, we use INTACT (Isolation of Nuclei Tagged in specific Cell Type) to generate neuronal-specific molecular atlases (transcriptome and chromatin landscape) of cocaine response. Additionally, we are examining cocaine responses in neurotransmitter and chromatin regulator mutants to identify the mechanisms driving cocaine-induced behavioral changes. Future research will integrate transcriptomic and chromatin accessibility datasets to achieve a detailed molecular characterization of cocaine exposure across distinct neuronal cell types, to ultimately elucidate the fundamental molecular mechanisms underlying cocaine-induced changes.

501B Optimization of Neuronal Nuclei Isolation for Molecular Profiling in *C. elegans*

Shelby Nyree Lauzon, Dalton Patterson, Sharrell Greene, Helen Zhou, HaoSheng Sun CDIB, The University of Alabama at Birmingham

Tissues within multicellular organisms are composed of diverse cell types, each playing a distinct role in tissue structure and function. Understanding cell-specific gene expression and function requires isolating individual cell types for analysis. INTACT (Isolation of Nuclei Tagged in specific Cell Type) enables the direct immunoprecipitation of tagged nuclei, eliminating the need for laborious and technically challenging flow cytometry sorting methods. The nervous system has higher cell type diversity compared to other tissue types, with the *C. elegans* nervous system composed of 118 distinct neuronal subtypes. Additionally, the *C. elegans* nervous system is considerably more difficult to isolate for molecular characterization than other tissue types. Here, we optimize methods for isolating *C. elegans* neuronal-specific nuclei and downstream molecular profiling. First, we optimized methods for reproducible and efficient nuclei isolation using a machine-based homogenization protocol rather than laborious manual homogenization across all post-embryonic developmental stages. This optimized method delivers more consistent homogenization, leading to increased reproducibility. Additionally, we are optimizing nuclei isolation for compatibility with snRNA-seq to improve transcriptomic resolution. Lastly, we are interrogating chromatin profiling accessibility (ATAC-seq) with nuclei obtained from our isolation method. Altogether, we present a refined protocol for *C. elegans* neuronal nuclei isolation, enabling high-resolution analysis of transcriptional and chromatin dynamics across development and experimental conditions. This optimized approach will enhance our understanding of molecular mechanisms underlying neuronal development and function.

502B Determining the role of paternal microRNAs in epigenetic inheritance in *C. elegans*

Alexis G. Leach[1], Grace Lee[1], Colin Conine[1,2]1University of Pennsylvania, [2]Children's Hospital of Philadelphia

Sperm was originally thought to contribute only genetic information to the next generation; however, recent studies show that epigenetic information is also transmitted to the egg during fertilization, eliciting non-genetically inherited phenotypes in progeny. Interestingly, the best characterized, causal carriers of this information are sperm non-coding, small regulatory RNAs (microRNAs and tRNA-fragments). In mice, sperm small RNAs have been demonstrated to transfer from soma-to-germline to regulate early development and inherited offspring phenotypes.

In *C. elegans*, endogenous small interfering RNAs (endo-siRNAs) in sperm can also affect offspring phenotypes transgenerationally, and further can be transferred from the soma to the germline. However, whether sperm miRNAs in worms are capable of transmitting inherited information and if this can be influenced by the soma has not been explored. To address this question, we have utilized temperature sensitive germline and inducible somatic miRNA depletion worm strains. Using these strains, we have demonstrated that gene expression in offspring sired by miRNA deficient fathers is altered throughout development. In adult progeny derived from miRNA depleted sperm, we find modulated expression of stress response and immune-related genes, indicating that potentially adaptive phenotypes are non-genetically encoded by sperm miRNAs. To further investigate the role of sperm miRNAs in offspring development, I plan to sequence embryos at each cell division using single-cell RNA sequencing. Additionally, I will challenge animals sired by sperm-miRNA deficient fathers with various insults (such as stress and immune assays) related to significantly altered genes in our dataset. Furthermore, I will measure gene expression and developmental changes of offspring sired by somatic-miRNA deficient fathers. The results from our studies show that sperm miRNAs are indeed implicated in non-genetic inheritance in *C. elegans*, thus revealing their conserved role in transmitting epigenetic information across generations from mammals to worms. This work establishes the worm as a powerful model to study mechanisms underlying how sperm miRNAs transmit environmentally regulated, heritable information across generations.

503B T-CLASS: an online tool for the identification and classification of physiological changes such as aging and longevity using transcriptome data

Seung-Chul J. Lee[1], Gee-Yoon Lee[1], Sieun S. Kim[1], Seokjin Ham[1], Jooyeon Sohn[1], Yunkyu Bae[1], Seong Kyu Han[2], Seung-Jae V. Lee[1]1Department of Biological Sciences, Korea Advanced Institute of Science and Technology, [2]Department of Biological Sciences, Inha University

The identification of the key molecular changes underlying physiological processes, including aging and longevity, from transcriptome data remains challenging. In this study, we present Transcriptomic CLassification via Adaptive learning of Signature States (T-CLASS), an online tool that identifies optimal gene sets of several hundred genes that provide minimal representation of biological states from transcriptome data. We systematically evaluated the effectiveness of T-CLASS across multiple species and conditions. We focused our analysis on the three representative longevity-promoting regimens in *Caenorhabditis elegans*, including reduced insulin/IGF-1 signaling pathway (rIIS), dietary restriction (DR), and reduced mitochondrial function (rMF), by performing transcriptome profiling and data integration of 27 publicly available RNA seq datasets. For rIIS, we obtained eleven different datasets of eight *daf-2(e1370)* mutants, one *daf-2(e1368)* mutant, one *daf-2* RNAi-treated animals, and one auxin-induced protein degradation (AID) strain targeting DAF-2. For DR, we analyzed eight datasets: three DR-mimetic *eat-2(ad1116)* mutants and two *eat-2(ad465)* mutants, and three directly diet-restricted animals. For rMF, we used nine datasets: two *isp-1(qm150)* mutants, one *clk-1(qm30)* mutant, one *nuo-6(qm200)* mutant, three *cco-1* RNAi-treated animals, one *mrps-5* RNAi-treated animals, and one *sod-2(ok1030)* mutant. We then showed that T-CLASS successfully classified transcriptomic changes caused by mutations with ambiguous association with the three longevity-promoting regimens, including germline-defective *glp-1(e2141)* mutant, sensory-defective *daf-10(e1378)* mutant and *osm-3(dh441)* mutant, and E3 ubiquitin ligase-defective *vhl-1(ok161)* mutant. In addition, T-CLASS accurately categorized ten lifespan-extending small molecules, including rotenone, metformin, allantoin, rapamycin, psora-4, D-glucosamine, rifampicin, JM03, atracurium, and monorden, into the three longevity-promoting regimens, among which we experimentally validated the effect of rifampicin as a proof of principle. T-CLASS also effectively distinguished the transcriptomic changes caused by different types of cellular senescence in mouse embryonic fibroblasts and by different human cancer categories. Overall, T-CLASS is an effective, user-friendly, and practical tool for uncovering and classifying physiological changes caused by genetic and pharmacological interventions based on transcriptome.

504B Elucidating chromatin remodeling mechanisms in learning and memory across development

Hee Kyung Lee, HaoSheng Sun Department of Cell, Developmental and Integrative Biology, The University of Alabama at Birmingham

Neural plasticity integrates gene expression, chromatin remodeling, and synaptic modifications to regulate learning and memory. Although neuronal numbers remain largely stable post-birth/hatch, synaptic connectivity and neuronal properties continue to change with maturation, shaping learning ability. However, the impact of these developmental changes on learning remains unclear. Recent studies (St. Ange et al., 2024; Sun et al., 2021) have reported stage-specific transcriptional shifts in the nervous system, including AWC chemosensory neurons, with pronounced changes occurring during the L4-to-adult transition. It remains unclear if learning ability varies across developmental stages and how these differences relate to stage-specific transcriptional changes, particularly in the context of chromatin remodeling factors that may differentially regulate these changes.

Focusing on the L4-to-adult transition, we investigate how chromatin remodeling influences aversive olfactory learning. This well-characterized associative learning paradigm provides a genetically tractable model to study learning-induced transcriptional and chromatin changes at single-cell resolution. We conducted a functional screen of 76 chromatin remodeler mutants (null or hypomorphic), identifying several candidates with significant roles in learning (e.g., *let-418* [CHD3 ortholog]). Notably, some chromatin remodelers exhibited distinct roles at either L4 or adult stages, suggesting that chromatin accessibility may differentially regulate learning depending on developmental stage.

Future studies will employ neuron-specific transcriptome and chromatin profiling to investigate how chromatin accessibility and transcriptional dynamics change in AWC and its synaptic partners during learning across developmental stages. By linking chromatin remodeling to developmental transcriptional dynamics, this study provides a framework for understanding the epigenetic regulation of learning across life stages, thereby offering insights into how post-mitotic neurons sustain plasticity.

505B WormPicker 2.0: A robotic system for high-speed automated genetic manipulation and analysis of *C. elegans*

Zihao (John) Li, Serge Alhalbi, Logan Kaising, Christopher Fang-Yen Department of Biomedical Engineering, The Ohio State University

Worm picking, perhaps the most basic procedure in *C. elegans* research, is a labor-intensive and low-throughput manual process that poses practical limits for many experiments, especially for those requiring large numbers of strains. An automated method for worm manipulation has potential to greatly accelerate *C. elegans* research. In previous work, our laboratory developed WormPicker, a robotic system capable of genetically manipulating *C. elegans* by imaging, phenotyping, and transferring animals on standard agar media. Here we describe WormPicker 2.0, a completely redesigned robotic system with enhanced speed, capacity, and functionality for high-throughput genetic manipulation and analysis. WormPicker 2.0 features a fast 6-axis articulated robotic arm equipped with tools for animal picking and plate handling, a motorized microscope for high-resolution imaging, and a storage system accommodating up to 250 barcoded agar plates. The new design increases the plate capacity by 1.7x while reducing the footprint to 0.75 m x 1.2 m, fitting standard benchtops. To manipulate *C. elegans* growing on agar plates, the robotic arm first transfers plates from the storage, removes lids from the plates, and transports them to the microscope stage. Next, the microscope and AI-driven machine vision systems track animals and characterize developmental stage, morphology, sex, expression of fluorescent reporters, and other phenotypes. Guided by machine vision and capacitive touch sensing, the robotic arm then selectively transfers individual worms using an electrically self-sterilizing wire loop. WormPicker 2.0 achieves a transfer rate of 13 animals per minute, a 4x improvement over the previous system. We also upgraded the system control software to enable the WormPicker 2.0 to autonomously carry out complex tasks. The new software contains a graphical user interface, a task manager, a plate information database, and a hardware controller. We are applying WormPicker 2.0 to perform experiments that would be challenging using manual methods, such as high-throughput aging assays, analysis of natural variations in drug response, studies of transgenerational inheritance, and a genetic screen for modulators of sleep behavior. By significantly accelerating worm genetic manipulation and analysis, WormPicker 2.0 moves closer to becoming a general-purpose tool for *C. elegans* laboratories.

506B Discovery of genes regulating stress-induced sleep in *C. elegans* by using the Sequence Kernel Association Test

Sreekar Kutagulla[1], Zihao (John) Li[1], Kerry LeCure[2], David Raizen[2], Christopher Fang-Yen[1]1Department of Biomedical Engineering, The Ohio State University, [2]Department of Neurology, University of Pennsylvania

In addition to regular healthy sleep, animals enter a state of sleep during illness. This adaptive behavior, known as sickness sleep, helps recovery. To elucidate the mechanisms of stress-induced sleep (SIS), we conducted a genetic screen for modifiers of UV-induced sleep in *C. elegans*. We used the Million Mutation Project (MMP), a library of 2,007 randomly mutagenized and fully sequenced *C. elegans* strains. We assayed the behavioral quiescence of day-1 adult animals over four hours following a 1,500 J/m² UV exposure using the multi-well WorMotel platform for 751 strains randomly selected from the MMP library. Using the sleep data obtained from these MMP strains and their genomic sequence, we used a linear regression-based method, Sequence Kernel Association Test (SKAT), to identify genes statistically associated with the SIS phenotype. We generated a ranking for 5,035 genes that have more than five non-synonymous alleles tested in our genetic screen, based on their statistical significance obtained from the SKAT analysis. This ranking highlighted a set of genes significantly associated with the SIS phenotype. The associated genes include genes that have been experimentally verified previously, such as *rom-4*, *dmsr-1*, and *ceh-14*, demonstrating the predictive power of our model. To identify novel SIS-regulating genes, we are now phenotyping SIS in animals carrying knock-out versions of the novel candidate genes identified by SKAT. Further exploration of these genes holds promise for enhancing our understanding of the genetic basis of sickness sleep.

507B Exploring the Role of Stress and piRNA Regulation in Germ Cell Immortality

Lu Lu, Matt Simon, Stephen Frenk, Maya Spichal, Shawn Ahmed Genetics, University of North Carolina at Chapel Hill

Healthy germ cells maintain themselves in a pristine unstressed state and can be transmitted from generation to generation, indefinitely. Understanding how this germ cell immortality is maintained could provide insights relevant to cell aging and potential rejuvenation of somatic cells. Germ cell immortality is promoted by several pathways, including telomerase and piRNA-mediated genome silencing. piRNAs mediate transcriptional silencing of many loci, such as transposons, and prevent inappropriate silencing of rDNA and histone loci that are important for maintaining the epigenomic integrity of germ cells. The loss of piRNA silencing pathway in *C. elegans* results in a transgenerational sterility phenotype, termed the Mortal Germline phenotype (Mrt). Our previous studies on *prg-1*/Piwi mutants suggest that small RNA imbalance underlying the *prg-1*/Piwi pathway disruption results in the transmission of hereditary stress that builds up across generations, ultimately leading to germ cell atrophy and sterility of late-generation mutants. We are studying the role of a potential stressor that may contribute to the Mrt phenotype in *prg-1*/Piwi mutants and are exploring the regulatory mechanism of this stressor.

508B The role of *miR-238* in healthy aging in *Caenorhabditis elegans*

San Luc[1], Laura Chipman[2], Amy Pasquinelli[11]Molecular Biology, University of California, San Diego, [2]University of California, San Diego

MicroRNAs (miRNAs) are a class of short non-coding RNAs that post-transcriptionally regulate gene expression in pathways that include development, aging and stress responses. We previously showed that loss of *C. elegans* miR-238 is associated with a shortened lifespan but could detect no longevity or stress phenotypes in animals lacking its related sister miRNAs, miR-239a or miR-239b, individually or in combination. To understand the biological function of miR-238 and how it differs from its highly related sisters, we generated reporter strains for analyzing the expression of miR-238 during development, aging and under stress. So far, we have observed predominant expression in the intestine, which prompted examination of *miR-238* mutants for defects in this tissue. Preliminary results indicate that loss of miR-238 results in a premature decline in integrity of the intestine. Additionally, we have found that *miR-238* mutants exhibit motility defects earlier in adulthood than wildtype animals. Thus, miR-238 promotes healthy aging. To identify targets whose mis-regulation in *miR-238* mutants might contribute to the reduced lifespan and healthspan phenotypes, we have performed transcriptomic profiling. Candidates that are up-regulated upon loss of miR-238 and have binding sites for the miRNA are currently being tested as potential direct and biologically relevant targets of miR-238 regulation in aging *C. elegans*. Overall, the goal is to provide more insight into how a single miRNA regulates specific targets to influence longevity and stress responses in an intact animal.

509B Using a Kabuki Syndrome model in *C. elegans* to screen potential therapeutics

Megan Lymburner[1], Nathaniel J Ortiz[2], Jaejoon Choi[2], Karolina Mizeracka[2], Eunjung A. Lee[2], Olaf Bodamer[3], Maxwell G. Heiman[21]Genetics, Boston Children's Hospital and Harvard Medical School, [2]Genetics, Harvard Medical School, [3]Genetics, Boston Children's Hospital

Kabuki Syndrome (KS) is a rare neurodevelopmental disorder arising from haploinsufficiency of the histone H3K4 methyltransferase KMT2D (90% of cases) or the histone H3K27 demethylase KDM6A (10% of cases), which normally promote active transcription by altering chromatin accessibility. KS is characterized by a diverse range of symptoms with variable severity including skeletal abnormalities, intellectual disability, developmental delays, and organ malformations. There are no current treatments for KS. Importantly, there are few laboratory models to study the effects of partial protein loss as occurs in KS, rather than complete gene knockouts. Here we develop a model of KS in C. elegans using degron-mediated protein depletion and demonstrate a quantifiable phenotype to assess the effects of potential therapeutics. Briefly, an auxin-inducible degron was inserted via CRISPR/Cas9 editing in set-16/KMT2D or utx-1/KDM6A, and varying concentrations of auxin (0.01 mM, 0.1 mM, 1.0 mM) were used to achieve controlled partial depletion of the tagged protein. While no overt phenotype was observed at intermediate protein levels, transcriptional profiling revealed thousands of genes that exhibited dose-dependent responses that are highly correlated with SET-16 and UTX-1 levels. The gene with the greatest response, pals-14, was used to create a transcriptional reporter strain (pals-14pro: GFP) that provides a quantitative fluorescent readout of the organismal response to SET-16 depletion. We adapted this reporter strain to a multi-well platform for automated imaging and analysis of fluorescence intensity of ~50 animals per well, allowing quantification of the response to SET-16 depletion over two days of larval development. In summary, this innovative C. elegans model of KS presents the opportunity to rapidly screen potential therapeutics that may improve residual protein activity or otherwise mitigate the effects of partial protein depletion in a whole-animal model.

510B Exploring the Relationship Between Ploidy and Fitness in Wild-Type and Mutant *C. elegans*

Tevon Madry, Gyorgyi CsankovszkiUniversity of Michigan

Polyploidization is a rare and seemingly paradoxical biological phenomenon. While polyploids may have a fitness advantage in acutely stressful environments, they tend to have lower fitness than diploids under normal conditions. We have maintained distinct diploid and tetraploid strains of *her-1 sdc-2 C. elegans* mutants, in which the tetraploids arose spontaneously, as well as distinct diploid and tetraploid strains of wild type *C. elegans*, in which the tetraploids were induced. Typically, *C. elegans* with an X:A ratio of 1:2 develop as males, while individuals with an X:A ratio of 1:1 develop as hermaphrodites. Our mutant populations only include worms that have a 1:2 X:A ratio, as the *sdc-2* mutation is lethal in worms with a 1:1 X:A ratio, due to lack of dosage compensation. Furthermore, a mutation in *her-1* yields worms that are anatomically hermaphroditic, while remaining karyotypically male. We measured the length and nuclei of these worms and found that tetraploid worms are longer than diploids, and that their nuclei are larger. Furthermore, using immunofluorescence microscopy, we show that the wild-type strains undergo dosage compensation, while the mutant strains do not. We also show that tetraploid autosomes associate in a pairwise fashion during meiosis I, and that the localization of a repressive histone modification localizes to the unpaired X regardless of the X:A ratio. We performed brood counts to compare the fitness of these strains under standard laboratory conditions and saw that the tetraploids had a significantly reduced fitness. We saw a different pattern when comparing the *her-1 sdc-2* mutant diploid and tetraploid worms in that the two ploidies had statistically similar fitness values. These mutants, however, had significantly lower fitness than both the diploid and tetraploid wild type animals. Interestingly, after a year of regular maintenance, we saw a significant increase in the fitness of these *her-1 sdc-2* mutants. We performed whole genome sequencing in order to investigate potential genetic alterations associated with this change in fitness, as well as any differences between ploidies. These findings shed light on the nature of polyploidy in *C. elegans* and its effect on fitness. Future work will elucidate potential genetic and or epigenetic alterations associated with increased fitness in *C. elegans* of differing ploidies, as well as respective cellular and molecular phenotypes.

511B Absence of R-Loops at the chromosomal breakage regions during programmed DNA elimination in the parasitic nematode *Ascaris*

Prabal Singh Maharjan, Brandon Estrem, Jianbin WangBiochemistry & Cellular and Molecular Biology, The University of Tennessee at Knoxville

The genome of most organisms remains constant throughout their life; however, some species undergo a process called programmed DNA elimination (PDE), where DNA loss is observed during germline to somatic differentiation. One notable example is *Ascaris*, a parasitic nematode that infects humans/pigs. In *Ascaris*, DNA double-strand breaks (DSBs) are generated to fragment the chromosomes. These DSBs reproducibly occur within 3-6 kb regions of the genome called chromosomal breakage regions (CBRs). How DSBs are targeted to the CBR remains a major unanswered question in *Ascaris* PDE. No conserved motifs or structural features have been identified to associate with the CBRs. However, analysis of chromatin accessibility at CBRs using ATAC-seq reveals that these regions are more accessible during PDE, suggesting an increase in accessibility may play a role in *Ascaris* PDE. Chromatin accessibility is often linked to transcription and replication. We hypothesize that transcription and/or transcription-replication collisions could generate R-loops, DNA-RNA hybrids, at the CBRs, leading to double-strand breaks (DSBs). To examine the role of R-loops in the DSB formation, we performed DRIP-seq to identify and quantify R-loop formation in the vicinity of the CBRs. Our data did not show an association between R-loops and the CBRs. However, we observed a strong enrichment of R-loops at the chromosome arms in early embryos. Intriguingly, the distribution of R-loops correlates with the density of single nucleotide polymorphisms (SNPs) across *Ascaris* chromosomes. In *C. elegans*, SNPs and hyper-divergent regions are concentrated on chromosome arms, suggesting that these features are maintained by long-term balancing selection. Additionally, R-loops are more enriched in chromosome arms in 32-64-cell embryos compared to 4-6-cell embryos, consistent with increased transcriptional landscape observed across these developmental stages. Overall, our study suggests R-loops are not a contributing factor that causes the DSBs at the CBRs during *Ascaris* PDE. Rather, the data indicates that *Ascaris* R-loops are associated with SNPs and suggests the R-loops may contribute to the genetic variations in the nematode genome.

512B FLInt 2.0: A high-precision method for transgene integration in *C. elegans*

Nawaphat Malaiwong, Michael O'Donnell Molecular, Cellular, and Developmental Biology, Yale University

Precise and efficient integration of transgenes is critical for genetic studies in *C. elegans*. The Fluorescent Landmark Interference (FLInt) method, introduced in 2023, provided a straightforward approach for integrating transgenic arrays using fluorescence markers such as tdTomato and GFP. However, its reliance on CRISPR/Cas9 led to false positives, requiring extensive F1/F2 screening to distinguish true integrations from the extrachromosomal arrays. Here, we present FLInt 2.0, an enhanced version of FLInt that significantly improves the accuracy and efficiency of transgene integration. By targeting a crRNA-recognition site within tdTomato, we generate a controlled landing site at the linker between tandem fluorescent sequences. This strategy exploits homology-directed repair (HDR) to restore tdTomato expression in non-integrated arrays, while non-homologous end joining (NHEJ)-mediated integration of plasmid DNA results in complete loss of tdTomato fluorescence. The contrast in phenotypic outcomes enables precise selection of integrated lines without F1/F2 screening. FLInt 2.0 enables transgene integration in *C. elegans* by introducing a built-in, fluorescence-based sorting mechanism, eliminating the need for labor-intensive worm picking, or generation of complex genetic backgrounds. Additionally, the incorporation of hygromycin selection further streamlines integrant isolation, making the method highly scalable and reproducible. This approach reduces screening time, enhances specificity, and minimizes false positives, positioning FLInt 2.0 as a powerful tool for functional genomics, genome engineering, and large-scale transgenic studies in *C. elegans*. We suggest that FLInt 2.0 will provide a robust and widely accessible solution for the *C. elegans* research community.

513B Assembly of the Dosage Compensation Complex on X Is Orchestrated by Self-Association of DCC Subunits and Dynamic Restructuring of Local Genome Architecture

Rajarshi P Ghosh[1], Yuri Malina[2], Connor D Luellen[2], Dhruv Sidhwani[2], Miki Okada[2], Zoe Yeh[2], Barbara J Meyer[21]Molecular & Cell Biology, HHMI/UC Berkeley, [2]Molecular & Cell Biology, HHMI / UC Berkeley

The *C. elegans* dosage compensation complex (DCC) equalizes X-linked gene expression between sexes by reducing transcription from both hermaphrodite X chromosomes, an essential process. Here we unravel the stepwise assembly of the DCC across X, revealing the molecular logic underlying its recruitment and function.

Our prior work showed that DCC recruitment relies on combinatorial clustering of distinct DNA motifs at *rex* sites (recruitment elements on X). We now show that chromatin accessibility plays a pivotal role in this process. Once bound, the DCC actively reorganizes surrounding nucleosomes.

STORM imaging shows that SDC-2, the key DCC recruitment factor, assembles into discrete, uniform clusters along X, while DPY-27, a condensin subunit, forms an amorphous point cloud with SDC-2 clusters embedded within. These results imply that SDC-2 forms a nucleation hub, while DPY-27 and other condensin subunits spread outward from multiple nucleation sites in an ATP-dependent manner.

The number of SDC-2 molecules inside clusters is invariant across protein concentrations, but cluster number scales with concentration, suggesting scaffold-limited clustering. To assess whether rex sites function as nucleation scaffolds, we used MINFLUX and STORM to visualize their spatial organization and Micro-C XL to quantify rex-rex interaction probabilities at nucleosome resolution. A subset of *rex* sites showed spatial coalescence, implying a role in scaffold-limited clustering of SDC-2. Unlike SDC-2, which forms stable hubs, DPY-27 transits rapidly between hubs, assembling into nanodomains that extend beyond edges of X chromatin, implying it forms a semi-constrained, ATP-dependent network that fluidly bridges chromatin territories. Optodroplet assays confirm DCC subunit self-association.

Using a 3D Convolutional Neural Network for analyzing high-throughput fluorescent images of X-bound SDC-2 and DPY-27, and entropy-based similarity analysis, we reconstructed the path of DCC assembly.

Using Micro-C XL and GRO-seq, we explored the impact of DCC-mediated chromatin remodeling on transcription. We found that X-chromatin is structured into distinct nested domains whose architectural state correlates with local transcription state. DCC depletion disrupts this organization, reversing domain types in sync with transcriptional shifts. Our results reveal a pivotal role for DCC in shaping chromatin topology and reinforce its impact on gene regulation.

514B An in-depth analysis of 3' untranslated regions in *Caenorhabditis elegans*

Emma Murari[1], Dalton Meadows[2], Marco Mangone[21]Arizona State University, Arizona State University, [2]Arizona State University

The 3′ untranslated regions (3′UTRs) of genes play a crucial role in gene expression by containing regulatory elements necessary for pre-mRNA 3′ end processing and post-transcriptional gene regulation. These regions influence mRNA stability, localization, and translation efficiency, making them essential for proper gene function. Despite their fundamental importance, 3′UTRs remain incompletely characterized in eukaryotic organisms, leaving significant gaps in our understanding of their diversity and regulatory potential.

To address this knowledge gap, we employed a comprehensive, multi-faceted approach to systematically extract, curate, and analyze 3′UTR sequences from publicly available transcriptomic datasets. Specifically, we examined 11,533 datasets encompassing the entire collection of *Caenorhabditis elegans* transcriptomes archived in the NCBI repository from 2009 to 2023. In addition to mining these extensive datasets, we implemented high-throughput cloning strategies to identify and validate rare 3′UTR isoforms, ensuring the inclusion of previously undetected transcript variants. Furthermore, we integrated and manually curated 3′UTR isoforms from previously published datasets, refining our understanding of the 3′UTR landscape in *C. elegans*.

The result of these efforts is an updated and significantly expanded version of the *C. elegans* 3′UTRome (v3), representing the most comprehensive 3′UTR resource available for any metazoan to date. This dataset encompasses 97.4% of the 20,362 experimentally validated protein-coding genes in *C. elegans*, providing refined and updated 3′UTR boundaries for 23,489 distinct 3′UTR isoforms. By leveraging this extensive dataset, we also identified and characterized key sequence elements involved in pre-mRNA 3′ end processing, shedding new light on the mechanisms governing mRNA maturation. Additionally, we utilized the updated 3′UTRome to refine microRNA (miRNA) target predictions, enhancing our understanding of post-transcriptional gene regulation.

This newly curated dataset has been incorporated in WormBase and represents a critical advancement in the study of 3′UTRs, offering a valuable resource for researchers investigating gene regulation, RNA processing, and transcriptome diversity in *C. elegans* and other eukaryotic systems. The insights gained from this work not only deepen our understanding of 3′UTR function but also provide a foundation for future studies exploring the roles of these regulatory regions in development, disease, and evolution. By making this dataset publicly available, we aim to facilitate further discoveries in RNA biology and gene regulation, underscoring the importance of 3′UTRs in shaping gene expression at the post-transcriptional level.

515B The SOSS complex as a factor of the piRNA biogenesis pathway

Joao Marques, Joana Pereirinha, Rene Ketting IMB Mainz

The SOSS complex is an essential catalytic domain of the Integrator complex, a key RNA polII regulator of transcriptional termination. While acting as part of the Integrator, the SOSS is essential for the transcription of small nuclear RNAs, as at these loci INTS-6 regulates the phosphorylation levels of PolII, stalling it at crucial promoter proximal locations, and allowing timely cleavage of pre-RNAs. In *C. elegans*, piRNAs form an important class of small RNAs and require Integrator for their transcription. Unfortunately, not much is known in the gap between pre-piRNAs' cleavage by Integrator until their loading into PETISCO, a germline specific complex that safeguards piRNA transcripts from degradation. In this work we share preliminary evidence that connects the SOSS complex to the piRNA pathway. In an attempt to find other PETISCO interactors, we conducted immunoprecipitation of TOST-1, a PETISCO interactor followed by mass-spectrometry analysis and identified all of *C. elegans* SOSS (*Ce*SOSS) subunits. *Ce*SOSS is an heterotetramer comprising two INTS-3 and NABP-1. While in its tetrameric form, this complex can also bind to the C-terminus of INTS-6. *Ce*SOSS differs from the canonical SOSS seen in other species, as it lacks a homologue of INIP, a deeply conserved and yet uncharacterized protein bound to INTS-3. AlphaFold 3 predicts an interaction between INTS-3 (CeSOSS) and ERH-2 (PETISCO) in the same structurally conserved INIP binding pocket. We will present ongoing work to further probe this tantalizing connection between the Integrator complex and piRNA precursor processing.

516B Rewriting the sequence of telomeric DNA

Benjamin McCarthy[1], Ambika Bhattarai[2], Will Tang[2], Evan Lister-Shimauchi[2], Julie Seohyun Lee[2], Yun Li[2], Daniel R Schrider[2], Shawn Ahmed[21]Genetics, UNC Chapel Hill, [2]UNC Chapel Hill

Telomeres are composed of tandem repeats, (TTAGGG)n in mammals and (TTAGGC)n in nematodes. Long-read genome sequencing data revealed that human and nematode telomeres were peppered with mutations, mostly insertion and deletion (indel) mutations, where the most frequent mutations in both species are T or G insertions at initial or terminal bases of telomere repeats, respectively. This implies that metazoan telomeres are mutated by a common biological process. Rare long insertions present in telomeric DNA of *C. elegans* strains were copied from internal segments of the genome, suggesting the template-shifting activity of DNA polymerase theta. Consistently, *C. elegans* pol theta is known to create small deletions bordered by G-rich tracts of DNA and is therefore likely to respond to G-quadruplex-mediated replisome blocks in telomeres (1).

Although telomeres of *C. elegans* strains grown in the lab for many years were densely peppered with mutations, recently isolated wild *C. elegans* strains possessed many kilobases of perfect TTAGGC telomere repeats, indicating that telomerase has exceptional fidelity in vivo. Multiple scaffolds for some telomeres reveal that telomerase and DNA polymerase theta compete to repair telomere damages. We found that telomeres mutate at a rate that is ~3,000-fold higher than that of non-repetitive internal segments of the genome, likely due to replication blocks that frequently occur during telomere replication. We conclude that telomeres are among the most polymorphic regions of metazoan genomes. Marked depletion of indel mutations from telomeres of wild *C. elegans* strains can be explained by forces of natural selection that do not impact *C. elegans* strains grown in the lab.

517B Strand Secrets: Uncovering Tissue-Specific Patterns behind microRNA Strand Selection Using the HiTmiSS Assay

Dalton Meadows, Amanda Ellis, Marco Mangone Arizona State University

MicroRNAs (miRNAs) are 16-24 nucleotide non-coding RNAs that mature to form semi-complementary duplexes. One strand of each duplex is loaded onto an Argonaute-like protein to regulate gene expression by targeting semi-complementary elements in the 3' untranslated regions (3'UTRs) of mRNAs. This process is highly conserved across metazoans, yet much about strand selection remains unknown. The identity of the 5′ nucleotide and thermostability of the duplex play some role, yet a significant amount of miRNA strand selection cannot be predicted using these metrics, suggesting additional patterns at play.

Here, we used a novel in-house High-Throughput miRNA Strand Selection (HiTmiSS) assay to track miRNA strand usage in all 190 *C. elegans* miRNAs throughout all six developmental stages. To deconvolve our results at the tissue level we optimized our HiTmiSS assay to detect miRNA strand selection in a tissue-specific manner. We first profiled the intestine and found patterns of miRNA expression that differed from the whole worm, suggesting the strand selection decision is regulated at the level of individual tissues. To further investigate this phenomenon, we modified our lab's dual color reporter plasmid construct to develop a two-color fluorescent reporter strain of *C. elegans*. These *C. elegans* express a green and a red fluorescent protein each under the control of a different strand of the miRNA *let-7* and allowed us to capture live images of strand selection in the intestine for the first time.

In conclusion, our research identified novel, important, and conserved patterns of miRNA strand selection throughout *C. elegans* development, correlating with previously observed developmental phenotypes. Specific to the intestine, our data both reinforced the tissue specificity of strand selection and revealed a novel phenotype upon depletion of both strands of *let-7* in the intestine. These findings highlighted novel structural principles underlying strand selection which can be applied to higher metazoans.

518B Homeostatic small RNA levels are essential for sperm-based fertility

Ha Meem[1], Alicia K Rogers[21]BIOLOGY, University of Texas at Arlington, [2]Biology, University of Texas at Arlington

RNA interference (RNAi) is a dynamic gene regulatory mechanism that requires appropriate production and balancing of different classes of small RNAs. The perinuclear *Mutator* focus houses the amplification machinery responsible for the production of several classes of small interfering RNAs (siRNAs). Thus, there is competition between these classes for resources within the *Mutator* focus. In our previous work, we identified a feedback mechanism required for maintaining homeostatic small RNA levels. This feedback motif relies on two 'siRNA sensor regions' (*sensor of siRNAs-1* (*sosi-1*) and *eri-6[e-f]*) encoded within the genome locus of *eri-6*, that monitor levels of different *mutator*-dependent small RNA classes and enables dynamic regulation of siRNA production. We showed removal of the *sosi-1 eri-6[e-f]* sensors does not disrupt the function or protein levels of ERI-6, a small RNA biogenesis factor, thus we have a novel paradigm for studying the role of RNAi homeostasis in fertility. In *C. elegans,* mutations in several RNAi factors are known to cause temperature-sensitive fertility defects ranging from immediate sterility to a progressive generational decrease in brood towards sterility. We tested whether disrupting RNAi homeostasis affects fertility. We performed a transgenerational heat stress brood size assay and found *sosi-1 eri-6[e-f]Δ* animals lay 51% fewer eggs, with increased rates of unfertilized eggs, compared to wild-type animals. This indicates reduced fertility is an immediate consequence of disrupting RNAi homeostasis. After fourteen generations of heat stress, *sosi-1 eri-6[e-f] Δ* animals became sterile. In the generations prior to the onset of sterility, we observed increased rates of males; however, the fertility of *sosi-1 eri-6[e-f]Δ* males was completely compromised. Our data suggest RNAi pathways' ability to maintain homeostasis by self-modulating small RNA levels is critical for RNAi function, particularly during stress, and is essential for sperm-based fertility.

519B Phenotypic Characterization of Mitochondrial Fission, Fusion, and Oxidative Phosphorylation Mutants *drp-1(tm1108)*, *fzo-1(tm1113)*, and *isp-1(qm150)* in *C. elegans* Sperm

Zahra Mohammad, Katherine Maniates, Aastha Lele, Nanci Kane, Jon Dietz, Chris Rongo, Andrew Singson Rutgers University

Mitochondria are required for the basic energetic requirements of cells. Sperm have a large energetic requirement because they are highly active and motile cells. In sperm, mitochondrial fission and fusion must be regulated to ensure that the sperm expend the optimal amount of energy required for spermiogenesis and subsequent fertilization. Mitochondria are dynamic organelles that undergo fission and fusion, and this characteristic controls their ability to produce ATP and free radicals. *drp-1* mediates mitochondrial outer membrane fission, while *fzo-1* and *eat-3* mediate mitochondrial outer membrane fusion. To further probe mitochondrial function, we are also examining mutants in the electron transport chain including, *isp-1* to determine if mitochondrial ATP generation is crucial for spermiogenesis. We hypothesize that mitochondrial regulation and ATP production is tied to sperm activation and the function of mature sperm. We are characterizing the impact of mutants *drp-1*, *fzo-1*, and *isp-1* to determine their impact on sperm activation and function. Our analysis shows that loss of *drp-1* causes deficits in sperm motility and impacts the fertility of both male and hermaphrodite *C. elegans*. We saw that *drp-1(tm1108)* male sperm is less competitive than wild-type sperm and are currently elucidating the extent of the sperm motility phenotype. Ongoing work will complete parallel analysis for *fzo-1* and *isp-1* mutants. We hypothesize that there will be a decrease in *drp-1(tm1108)* and *fzo-1(tm1113)* male sperm activation compared to wild-type *C. elegans*, and that *fzo-1* and *isp-1* male sperm will have a deficit in their activation and interaction with wild-type hermaphrodites. Through this phenotypic analysis, we are gathering the data necessary to understand how mitochondrial dynamics impact spermiogenesis in *C. elegans.*

520B Femtosecond laser microdissection for isolation of regenerating *C. elegans* neurons for single-cell RNA sequencing

Peisen Zhao, Sudip Mondal, Adela Ben-Yakar The University of Texas at Austin

Understanding nerve regeneration requires uncovering the molecular activities driving this process at single-neuron resolution in model organisms like *C. elegans*. However, current cell isolation techniques cannot selectively isolate neurons based on specific regeneration phenotypes in *C. elegans*. Here, we introduce femtosecond laser microdissection (fs-LM), a single-cell isolation method that uses the micrometer-scale precision of fs-laser ablation to dissect individual cells directly from living tissue. We demonstrate that fs-LM enables sensitive and specific gene expression profiling through single-cell RNA sequencing (scRNA-seq), while minimizing stress-related transcriptional artifacts typically induced by tissue dissociation. Applying scRNA-seq to fs-LM-isolated regenerating neurons uncovered distinct transcriptional programs associated with successful and failed regeneration in wild-type and dlk-1(0) mutants, respectively. This approach also revealed transcriptional heterogeneity within the same neuron type, identifying gene modules whose expression patterns correlate with axon regrowth rates. These findings establish fs-LM as a powerful, spatially resolved single-cell isolation method for mapping phenotype-to-genotype relationships.

521B Perturbation of body size genes via RNAi-by-feeding in *C. inopinata*

Kimberly Moser[1], Gavin Woodruff[21]Biology, University of Oklahoma, [2]School of Biological Sciences, University of Oklahoma

Body size is a fundamental organismal trait, and the genetic basis of body size variation remains largely unknown. The nematode *Caenorhabditis elegans* is a widely studied model organism with a sophisticated experimental genetic toolkit in tandem with a wealth of developmental genetic background knowledge. *Caenorhabditis inopinata* is a sister species of *C. elegans* that has evolved a much larger body size. Several genes have been shown to regulate body size and shape in *C. elegans*, and these genes represent prime candidates as substrates for the evolution of large body size in *C. inopinata*. Here, we perturbed a number of genes associated with this pathway in both species: *dbl-1*, *lon-1*, *lon-2*, and *lon-3* (in addition to three essential actin-encoding genes). Actin gene knockdown reliably promotes inviability in both species. Conversely, the targeting of known body size genes via RNAi-by-feeding had minimal impacts on *C. elegans*. *dbl-1(RNAi)*yielded no obvious impacts on body size in both species, likely due to its neuronal expression. *lon-1*, *lon-2*, and *lon-3* knockdown promotes elongated body sizes in *C. inopinata*, consistent with functional conservation of these genes across species with divergent body sizes. Ongoing work assessing the transcriptional abundance of target genes following RNAI (as well as the targeting of other body size genes) will also be presented. With the emergence of reliable CRISPR/Cas9-mediated target mutations, the idiosyncrasy of RNAi-by-feeding results across tissues, strains, and species is likely to be mitigated. Regardless, these preliminary results reveal potential functional conservation of body size genes across species with radical changes in body shape and size.

522B Dynamic association of the piRNA transcription complex with genomic piRNA clusters

Victoria Murphy, Anna Vakhnovetsky, John K Kim Biology, Johns Hopkins University

The PIWI-interacting RNA (piRNA) pathway is conserved across metazoans and plays an essential role in germline development and fertility. In *C. elegans,* the PIWI Argonaute PRG-1 binds individual piRNAs within the piRNA-induced silencing complex to silence target transcripts. The >15,000 unique piRNAs encoded in the *C. elegans* genome are clustered within two regions on chromosome IV: the “small” (2.5 Mb) and “large” (3.7 Mb) clusters. Transcription from these clusters requires the ancient snRNA-activating protein complex (SNAPc/SNPC), which localizes to large foci at the periphery of germline nuclei as part of the larger piRNA transcription complex, the USTC (Upstream Sequence Transcription Complex). ChIP-seq data of the core SNPC factor SNPC-4 suggest that piRNA SNPC binds both piRNA clusters simultaneously. However, bulk ChIP-seq methods cannot resolve SNPC binding dynamics within individual germline nuclei, thus limiting our understanding of how SNPC recruitment to each piRNA cluster may be differentially regulated during germline development. The frequency of SNPC association with each cluster remains unknown. To address this, we used DNA FISH and immunofluorescence to measure the localization of SNPC and piRNA clusters in fixed germline tissue. Preliminary data indicates that SNPC foci preferentially associate with the large piRNA cluster, suggesting that binding between SNPC and the piRNA clusters is dynamic, with more transient SNPC association with the small cluster. We are also developing live-imaging methods to track SNPC binding dynamics in real-time. Investigating how SNPC foci associate with piRNA clusters throughout the developing germline may reveal a novel mechanism regulating piRNA transcription.

523B A major regulator of germline transcription, LSL-1, contributes to developmental delay when histone methylation is inappropriately inherited

Benjamin Nguyen, Zaynab Massenburg, Brandon Carpenter Molecular and Cellular Biology, Kennesaw State University

Histone methylation is a post-transcriptional modification to the N-terminal tails of histone core proteins that regulates DNA accessibility, and consequently, gene expression. Like DNA, histone methylation can be inherited between generations and is highly regulated during embryonic development. At fertilization, histone methylation undergoes maternal reprogramming to reset the epigenetic landscape in the new zygote. During maternal reprogramming of histone methylation in the nematode, *C. elegans*, H3K4me1/2 is removed by the H3K4 demethylase, SPR-5, and H3K9me1/2 is subsequently added by the histone methyltransferase, MET-2. Maternal reprogramming by SPR-5 and MET-2 is antagonized by the H3K36 methyltransferase, MES-4, which maintains H3K36me2/3 at germline genes to ensure proper germline gene expression in germ cells. In the absence of SPR-5; MET-2 maternal reprogramming MES-4 aberrantly maintains H3K36me2/3 at germline genes in the soma leading to somatic expression of germline genes and a range developmental phenotypes including developmental delay. Recent work identified a germline transcription factor, LSL-1, that regulates germline gene expression during development. From our own immunohistochemistry and transcriptional analyses performed on *spr-5; met-2* mutant progeny, we find that *lsl-1* is significantly upregulated in somatic tissues and that germline genes regulated by LSL-1 overlap with genes that are upregulated in the somas of *spr-5; met-2* mutants. Together, these data suggest that LSL-1 may maintain expression of germline genes aberrantly in somatic tissues and contribute to developmental delay in the absence of SPR-5; MET-2 maternal reprogramming. To test this hypothesis, we knocked down LSL-1in *spr-5; met-2* mutants using RNA interference (RNAi) and find that the somatic expression of MES-4 germline genes is significantly reduced which correlates with a partial rescue of the severe developmental delay. Together, our findings suggest that when histone methylation is inappropriately inherited the germline transcription factor, LSL-1, contributes to developmental delay by maintaining high levels of germline gene expression aberrantly in somatic tissue. Furthermore, our data implicates germline transcription factors as potential culprits that may help exacerbate soma-to-germline conversions that occur in human neurodevelopmental disorders that arise due to mutations in transcriptional repressor complexes.

524B Comparative genomics approach identifies TRPA-1 ion channel as a regulator of pathogen avoidance

Martin D. Nicholas, Mohammad Tanha, Yiting Xu, Ji Zhang, Laura D. Mydlarz, Mark W. Pellegrino Biology, University of Texas at Arlington

Coral reefs are under increasing threat due to rising ocean temperatures and pathogen infection. Strategies to help protect threatened coral populations are warranted considering their ecological and economic importance. However, studying the mechanisms of coral responses to stress is challenging due to the lack of available genetic approaches and the difficulty in rearing these marine organisms. To overcome this barrier, we employed a comparative genomics approach using *C. elegans* to predict coral host factors that may be necessary for protection against pathogen infection. We leveraged previously generated transcriptomic datasets analyzing the response of six coral species during infection with white plague disease. We identified 141 genes that were differentially expressed across two or more coral species, 89 of which were conserved in *C. elegans*. By referencing available transcriptomic datasets of *C. elegans* responses to infection, we identified 17 candidates that were differentially expressed following pathogen challenge. We then performed a functional screen of these 17 candidate genes using loss-of-function mutants and measuring their survival during infection with the pathogens *Pseudomonas aeruginosa* or *Enterococcus faecalis*. Using this approach, the gene *trpa-1* was identified as a host factor promoting survival during infection. TRPA-1 is orthologous to mammalian TRPA1, a cation channel which acts as a sensory receptor for various environmental stimuli. Interestingly, we observed that the *trap-1* loss-of-function mutant was defective in avoiding *P. aeruginosa* and *E. faecalis*, suggesting that this behavioral defect is the cause of their increased susceptibility to pathogen infection, which we are currently exploring. Our study thus demonstrates the power of cross-species methods in identifying shared regulators of cellular response pathways.

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526B Creation and manipulation of transgenes using recombinases

Michael Nonet Department of Neuroscience, Washington University Med School

Transgenes are a critical part of the *C. elegans* research toolkit to examine a variety of cellular processes from neuronal activity to induction of stress responses. I have developed 3 methods that use site-specific recombinases to catalyze insertion or manipulation of sequences in the genome.

Recombination Mediated Cassette Exchange (RMCE) uses FLP recombinase to swap an injected large DNA fragment flanked by FRT and FRT3 sites with a similarly flanked landing site in the genome (Nonet, 2020, 2023). The event is designed to change a fluorescent marker associated with the landing site and simultaneously excise the recombinase expressing sequences. The insertions are typically selected for using Hyg or Neo resistance. Eight days after injection, animals homozygous for the insertion are identified based on fluorescent markers. Subsequently, the selection cassette can be excised by crossing to integrated Cre lines also developed using RMCE. Using this approach, my lab has created over two hundred transgenes to develop bipartite reporter systems (Nonet, 2020, 2024; Knobel et. al 2024). Efficiency in our hands is ~1 insertion per 2 injected animals and fidelity is exceedingly high.

Recombinase Mediated Insertion (RMI) uses phiC31 and FLP recombinases to integrate an entire circular DNA at an *attB* or *attP* containing landing site. Co-expression of FLP is required to de-concatenate the large arrays that form after introduction of DNA into the germline. RMI was developed to facilitate the creation of linked bipartite drivers and reporters (Nonet, 2024). However, several landing sites designed to use RMI as a general integration method have now been developed. The efficiency of RMI is ~10-fold higher than RMCE. In our hands, virtually every animal injected yields multiple independent integration events.

Recombination Meditated Homolog Exchange (RMHE) was developed to facilitate the use of bipartite reporters in complex genetic crosses (Nonet, 2024). Typically, driver lines expressing a transcription factor (e.g. GAL4) and reporter lines expressing a reporter (e.g. UAS GFP) are integrated at distinct positions in the genome. Creating a stable strain homozygous for a driver, reporter and a mutation involves segregating three loci. RMHE is a method that permits one to recombine two insertions (e.g. a driver and a reporter) integrated at the same insertion site in the genome using phiC31. *attP* and *attB* sites 5› and 3› of the insertions permit the recombination of the two transgenes from a *trans* to a *cis* configuration at an efficiency of about 10% in a germline phiC31 expressing background. The approach can similarly be used to recombine green and red transgenes integrated at the same position in the genome.

The poster will provide overviews of all three approaches, discuss strengths and limitations, lists of available tools, as well as a summary of ongoing efforts to further improve the systems.

527B Uncovering RNAi and chromatin modifying pathway co-regulation that protects germ cell identity during heat stress.

Favour E Nwose, Alicia K Rogers Biology, The University of Texas at Arlington

Robust gene regulation is important for cellular homeostasis, particularly during stressful environmental conditions such as elevated temperatures that adversely affect germ cell identity and fertility. RNA interference (RNAi) pathways and chromatin modification pathways are both important for gene regulation, resulting in proper cellular and developmental programs. We seek to understand how RNAi pathway and chromatin modification pathways collaborate in maintaining germ cell identity and fertility under stressful conditions in C. elegans. Work from our lab and others found that nuclear RNAi mutants experience a mortal germline (mrt) phenotype and exhibit sterility after several generations of being exposed to heat stress (25˚C). However, why each mutant reaches sterility at a different generation remains unclear. Multi-omic analyses (mRNA-seq, small RNA-seq, and Assay for Transposase-Accessible Chromatin (ATAC)-seq) from two RNAi mutants *mut-16* (pk710) and *hrde-1*(tm1200) showed that the onset of the mrt phenotype corresponds with aberrant expression of somatic and spermatogenesis-related genes within the germline that correlated with genome-wide increase in chromatin accessibility, resulting in loss of germ cell identity. Previous studies carried out in D. melanogaster have shown similar aberrant gene expression and loss of germ cell identity in mutants for a factor that links the small RNA and chromatin modifying pathways. This suggests the mechanisms by which RNAi and chromatin modifying pathways co-regulate gene expression to maintain germ cell identity may be evolutionarily conserved. Here, we aim to first establish if the onset of heat stress-induced sterility in each of six RNAi and chromatin modifying pathway mutants is triggered by defects in oocytes and/or sperm. Then we will determine the same subset of somatic and spermatogenesis genes that are dysregulated specifically during the generation at which heat stress-induced sterility manifests. With future experiments that assess changes in the chromatin landscape, we will advance our understanding of how RNAi mechanisms and chromatin-modifying pathways co-regulate gene expression, preserving germ cell identity and overall fertility of the animal during stress.

528B An intrinsically disordered region of Drosha selectively promotes miRNA biogenesis, independent of tissue-specific Microprocessor condensates

Bing Yang[1], Brian Galletta[2], Nasser Rusan[2], Katherine McJunkin[31]National Institute of Diabetes and Digestive and Kidney Diseases, [2]National Heart, Lung and Blood Institute, [3]Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases

Precise control of miRNA biogenesis is of extreme importance, since mis-regulation of miRNAs underlies or exacerbates many disease states. The Microprocessor complex, composed of DROSHA and DGCR8, carries out the first cleavage step in canonical miRNA biogenesis. Despite recent advances in understanding the molecular mechanism of Microprocessor, the N-terminal region of DROSHA is less characterized due its high intrinsic disorder. Here we demonstrate that Microprocessor forms condensates with properties consistent with liquid-liquid phase separation (LLPS) in select tissues in C. elegans. While DRSH-1/Drosha recruitment to granules is only partially dependent on its intrinsically disordered regions (IDRs), one of these N-terminal IDRs is crucial for biogenesis of a subset of miRNAs and normal development. A cis region of an IDR-dependent miRNA confers IDR-dependence to another miRNA, suggesting that the IDR recognizes sequences or structures in the miRNA primary transcript. Future studies will further elucidate the specificity of this interaction and the putative role of Microprocessor condensates.

529B A Guide RNA-Deficient Argonaute Reveals Spatial Coupling of Nuclear piRNA Transcription and Perinuclear Processing in *C. elegans*

Humberto J. Ochoa, Daniel J. Durning, Siyuan Dai, Craig C. Mello RTI, UMass Med

In *C. elegans*, Argonautes and their small RNA co-factors function within perinuclear nuage to regulate germline mRNA transcripts. We investigate the impact of a Y613E mutation in the Argonaute WAGO-1, which disrupts guide RNA binding. WAGO-1(Y613E) fails to silence targets, loses nuage enrichment, and instead exhibits increased binding to the VASA homolog GLH-1. In Y613E mutants, GLH-4 and the PIWI Argonaute PRG-1 co-localize within an enlarged nuage domain. Notably, this domain forms adjacent to intranuclear piRNA-cluster transcription sites, suggesting a spatial link. Loss of *prde-1*, required for piRNA transcription, prevents this domain's formation. Molecular data support a model in which GLH-4 (and, to a lesser extent, GLH-1) binds the 3' ends of piRNA precursors to facilitate decapping, trimming, and loading onto PRG-1. Our findings suggest that active piRNA transcription directs the proximal formation of a specialized nuage domain that enables piRNA maturation.

530B Alternative Splicing Contributes to Nervous System Maturation in *C. elegans*

Dalton C Patterson, HaoSheng Sun Cell, Development, and Integrative Biology, University of Alabama at Birmingham

Alternative splicing (AS) is a post/co-transcriptional process that produces multiple mRNA isoforms from a single gene via splicing factors (SF) complexes. AS affects approximately 90-95% of human protein-coding genes, generating diverse isoforms that expand the functional complexity of the genome. After birth, post-mitotic neurons undergo functional and anatomical changes guided by tightly regulated genetic programs, resulting in behavioral changes. AS's influence on gene regulation contributes to nervous system maturation. However, this direct regulatory mechanism affecting neuronal isoform expression across development remains to be studied. Rbfox1 (ortholog to fox-1 in C. elegans) is an evolutionarily conserved splicing factor implicated in neurodevelopmental diseases. Here, I am using the C. elegans model to investigate the role of fox-1 in AS regulation during postembryonic neuronal maturation. First, I am mapping the expression of fox-1 across the postembryonic development in single-neuron resolution using an endogenous Green Fluorescent Protein (GFP) reporter. Concurrently, I am utilizing a fox-1 knockout mutant to evaluate the functional consequences of losing fox-1 splicing on the maturation of locomotion across development. To further investigate fox-1 regulation and expression, I have accessed publicly available C. elegans short-read single-cell/nucleus RNA sequence data from two developmental stages: L4 (analogous to adolescence/puberty) and adult. Leveraging this data, I will evaluate fox-1 expression to identify neuronal cell types with the highest expression levels and map splicing factor regulatory networks involving fox-1 and its target isoforms. Given the limitations of short-read sequencing, I am also generating single-nucleus long-read RNA sequencing data from neuronal nuclei of control and fox-1 mutant animals across development. In addition to fox-1, I will apply this approach to additional conserved splicing factors (e.g., unc-75/CELF,exc-7/ELAV) to further evaluate how AS contributes to nervous system maturation. Altogether, this will significantly enhance our understanding of AS's role in neuronal maturation and uncover new insights into the genetic mechanism underlying NS development and the pathogenesis of neural disorders.

531C Multigenerational Adaptation to Novel Food Sources in *C. elegans*

Alexandria B Pete, Craig P Hunter Molecular & Cellular Biology, Harvard University

The natural life cycle of free-living nematodes follows a boom-and-bust pattern, with rapid population growth when resources are plentiful, followed by dispersal as resources deplete. This ecological pattern inspired us to examine how adaptation to a bacterial diet could serve as an effective model for studying multigenerational adaptation. In this study, we demonstrate non-genetic adaptation to dietary changes over multiple generations. When *C. elegans* is switched to a new bacterial diet, initial broods are small, but fecundity typically normalizes within 4-9 generations. Notably, adaptation to the new diet often results in maladaptation to the original food source. Crosses between nematodes adapted to different bacterial strains reveal that sperm and oocytes transmit distinct adaptive information to their progeny. These results suggest the presence of a flexible, heritable mechanism that enables rapid and reversible adaptation to fluctuating environmental conditions. While much research has focused on the persistence of a response to transient events («memory»), we propose that emphasizing heritable adaptation to likely future conditions - rather than memory of past events - could offer deeper insights into the evolutionarily selected epigenetic mechanisms driving these processes.

532C Neuron-specific Transcriptomic Changes in Response to Cocaine in *Caenorhabditis elegans*

Nico Pinzon[1], Jacob Suchman[1], Rachid El Bejjani[21]Davidson College, [2]Biology, Davidson College

Despite extensive research on the neurobiology of cocaine addiction, inhibiting its known mechanisms of action has proven insufficient for effective treatment. Our recent paper showed that cholinergic neurotransmission plays a key role in mediating cocaine-dependent egg-laying in Caenorhabditis elegans, suggesting that these neurons may undergo specific transcriptomic changes in response to cocaine. This study aimed to characterize these changes and identify potential regulatory mechanisms responding to cocaine.

To achieve this, we treated C. elegans with cocaine and used cellular dissociations followed by FACS sorting to isolate cholinergic neurons. We then performed RNA sequencing on these sorted neurons from both control and cocaine-exposed animals. Differential expression (DE) analysis using DESeq2 identified 152 DE genes (adjusted p-value < 0.05), with 119 upregulated and 33 downregulated DE genes in response to cocaine. To identify clusters of co-regulated DE genes, potentially regulated by a mechanism activated in response to cocaine, we binned genes based on LFC and clustered using hierarchical density-based clustering (HBDSCAN) and Clust clustering. HBDSCAN revealed two tight clusters of downregulated genes, while Clust revealed five distinct clusters of co-regulated upregulated genes.

To investigate regulatory mechanisms within the clusters, we extracted transcriptional regulatory regions (TRRs) and performed motif discovery using XSTREME and HOMER, followed by motif alignment with TomTom. We identified eight motifs significantly present in our clusters, including one novel motif and seven that match known C. elegans transcription factor (TF) binding sites. Upregulated genes were enriched for TF binding sites linked to well-described detoxification and stress response programs, while three motifs in TRRs of downregulated genes aligned with TFs implicated in neuronal function. Notably, our broadest cluster of DE genes appears to be co-regulated by a TF with a described broad functionality.

These findings provide insight into neuron-specific transcriptional responses to cocaine and suggest potential regulatory mechanisms that could be further explored as therapeutic targets for cocaine addiction. Future work will focus on experimentally validating these mechanisms to better understand their role in addiction biology.

533C A role for long non-coding RNAs in calcium signaling during cell migration and behavior

Vida Praitis, Julia Tlapa, Ezra Praitis Hill, Caleb Lee, Haonan Sun, Sam Kubica Biology, Grinnell College

Cellular calcium concentrations in the cytoplasm and in secretory stores are both dynamic and tuned, responding to the actions of calcium channels and pumps, including the conserved secretory pathway calcium ATPases (SPCA). In *C. elegans*, PMR-1/SPCA1 is required in oxidative stress response (Cho 2005), maintaining calcium homeostasis during heat stroke (Kourtis 2012), synuclein-related Ca++ cytotoxicity, (Buttner 2013), regulating oligosaccharide structure (Schifano 2019), and for cell migration during embryogenesis (Praitis 2013). To identify genes that act with *pmr-1* in cellular calcium homeostasis, we carried out a forward genetic suppressor screen of the *pmr-1(ru5ts)* embryonic lethal phenotypes and identified the *kez13* allele. Whole genome mapping, sequencing, and complementation analysis identified *kez13* as a 5 kb deletion upstream of *sma-9*. This genomic region contains high-occupancy transcription factor binding sites (Liang, et al 2003; Chen, et al, 2014) and two long non-coding RNAs, *linc-3290* and *linc-3291*(Akay 2019), which are conserved between *C. elegans* and *C. briggsae*. We used Crispr to generate two additional lines, *linc-3290(kez24D)* and *linc-3291(kez23D),* which delete each gene. While none of these alleles reduce embryonic viability on their own, each allele partially suppresses *pmr-1(ru5ts)* lethality, with strongest suppression when both genes are deleted. Introduction of additional copies *linc-3290* and *linc-3291* via a transgene enhance embryonic lethality in *pmr-1(ru5)* strains grown at permissive temperatures, indicating the relationship is dosage sensitive. *linc-3290* and *linc-3291* transcriptional fusions are expressed in embryos, including prior to enclosure, with stronger expression after the comma stage. We identified several additional calcium-related phenotypes in strains with deletions of *linc-3290* and *linc-3291*. *linc-3290(kez24)* males are not sterile, and have normal ray structure, but adult males curl into and remain in a cinnamon roll shape during male mating reversal. *linc-3290(kez24)* hermaphrodites have normal timing but reduced frequency egg-laying, while *pmr-1(ru5); linc-3291(kez23)* strains have the opposite phenotype and are precocious egg layers. *linc-3290* and *linc-3291* transcriptional fusions are expressed in nerve cells in the head, in the pharynx, in the male tail, in some body wall muscles, and in the vulva, consistent with a model whereby these genes are impacting calcium signaling in nerves and muscles.

534C The evolutionary history of nematode Argonautes

Lucas Prescott, Craig MelloRNA Therapeutics Institute, UMass Chan Medical School

Two paralogous lineages of Argonautes, AGOs and PIWIs, are inferred to have diverged around the last eukaryotic common ancestor (LECA) and are often differentiated by the source of their small RNA guides. Within eukaryotes, the branching order of some Argonaute clades has remained unsolved due to long branch attraction, e.g., the expanded group of worm-specific Argonautes including the catalytically dead WAGOs and the highly conserved but seemingly unloadable MED13 homologs. Our analysis of thousands of Argonaute sequences allowed us to resolve the roots of several clades, namely that all worm-specific Argonautes and the MED13 clade branch within the AGOs.

High-throughput structure prediction subsequently allowed us to explain how the worm-specific lineages evolved to accommodate guides produced by neofunctionalized RNA-dependent RNA polymerases (RdRPs). For example, the WAGO and CSR clades shared an ancestor with a MID domain Y to H substitution that enabled accommodation of 5′ triphosphorylated guides. The other nematode Argonautes ERGO-1 and ALG-3/4 are also loaded with RdRP-derived guides, yet they preserved the Y in their MID domain and instead depend on the RNA phosphatase PIR-1 to process their guides before loading. Although this Y is conserved in nearly all other eukaryotic Argonautes, the fact that most prokaryote Argonautes contain an H should alter how we imagine Argonautes' ancestral functions.

In addition to full-length Argonautes, we characterized individual Argonaute domains which were fused to other three other proteins: the Dicer homolog DCR-1, the worm-specific PETISCO subunit PID-3, and a factor required for RNAi inheritance ZNFX-1. Although Dicer's PAZ domain was acquired around the LECA, PID-3 and ZNFX-1 acquired MID domains from the nematode PRG-1 and CSR-1 Argonaute lineages, respectively.

Beyond monomers, structure prediction can be used to screen for direct interactors. Experimental datasets (Y2H, IP-MS, or proximity labeling) can be run on a single GPU within a few days, else the whole proteome can be pre-screened by how much coevolution occurs with a given bait protein. False positive "hallucinations" can be removed by requiring that multiple prediction methods and species all return similar multimers, i.e., technical and biological replicates. In addition to the Argonautes, we have screened dozens of proteins across the small RNA pathway and intend to expand this analysis to the whole interactome scale.

535C The Molecular Implications of Tetraploidy on Gene Expression

Angelica Previero, Györgyi Csankovszki University of Michigan

Polyploidy is a highly widespread phenomenon, appearing in processes such as evolution, cancer, regeneration, and development, sometimes in all cells which are part of a tissue, while other times in only a subset. However, even though polyploidy is clearly a fundamental phenomenon across life, the gene expression differences between diploid and polyploid cells are not clear. One of the few organisms that can be fully turned tetraploid is the roundworm Caenorhabditis elegans. Tetraploid C. elegans develop more slowly, are less reproductively fit, age faster, and have an overall shorter lifespan. Besides physiological differences, RNAseq data also shows differential gene expression in tetraploid C. elegans with respect to their diploid counterparts. Interestingly, several genes that are both upregulated and downregulated are tissue specific genes involved in tissue specific programs. Our hypothesis is that the lower surface-to-volume ratio affects the interactions between the DNA and the nuclear lamina, consequently affecting gene expression. Physiological and sequencing data about otherwise wild type diploid and tetraploid worms as well as diploid and tetraploid cec-4 mutants elucidate the implications of tetraploidy and the molecular basis of such consequences. Furthermore, this data highlights the effects of multiple copies of DNA on specific genes and starts to elucidate why this not always advantageous phenomenon is so conserved across different organisms and stages of life.

536C Conserved function of *rnt-1* in *Caenorhabditis elegans* and its sister species *Caenorhabditis inopinata*

Md Masudur Rahman, Gavin Woodruff School of Biological Sciences, University of Oklahoma

Body size is a fundamental phenotypic trait linked to fitness, metabolic rate, fecundity, and evolutionary history. *Caenorhabditis inopinata*, a sister species of *Caenorhabditis elegans*, exhibits a significantly larger body size, which led us to investigate the genetic diversity driving this phenotypic divergence. Transforming Growth Factor-β (TGF-β) superfamily ligands regulate many aspects of cell identity, function, and survival in multicellular animals, and a canonical TGF-β pathway regulates body size in *C. elegans*. A transcription factor connected to this pathway in *C. elegans*, *rnt-1*, exhibits divergent transcriptional dynamics during *C. inopinata* development. *C. elegans rnt-1* loss-of-function mutants have been reported to show reduced body size, suggesting a potential role of *rnt-1* in the evolution of body size in *C. inopinata*. Moreover, male tail defects were also reported due to the loss of *rnt-1* function.

To elucidate the function of *rnt-1*, we generated null deletion mutants in both species using CRISPR/Cas9 technology. Preliminary results reveal that *C. inopinata rnt-1* mutants are smaller than their wild-type counterparts, supporting the hypothesis that *rnt-1* regulates body size. Additionally, disruption in male tail morphology is observed in *C. inopinata rnt-1* mutants. These findings indicate the conserved function of *rnt-1* for body size regulation and male tail defects in *C. inopinata*. Developmental stage timing and individual fecundity rate assessments are ongoing. Ongoing assessments aim to confirm these findings in *C. elegans*.

Future investigations will be conducted to identify downstream targets, spatial gene expression of *rnt-1*, and the impact of *rnt-1* on seam and neural cell fate. This research will advance our understanding of the molecular mechanisms underpinning body size evolution and developmental plasticity in nematodes, offering insights into how transcriptional regulation can drive phenotypic diversity between closely related species.

537C Investigating Argonaute-small RNA binding specificity in *C. elegans*

Sanjana Rajeev[1], Carolyn Phillips[21]Molecular and computational biology, University of southern California, [2]University of southern California

RNA silencing is a fundamental mechanism by which gene expression is regulated. This pathway has two main players: small RNAs and Argonaute proteins. Small RNAs are short non-coding RNA molecules about 18-30 nucleotide in length and Argonaute proteins are the effector molecules which act as molecular guides to target and silence messenger RNAs (mRNAs). *C. elegans* have about 19 functional Argonaute proteins and multiple classes of endogenous small RNAs, including siRNAs, miRNAs, and piRNAs. Among these, 22G-RNAs represent a major class of secondary siRNAs that can be further divided into two subclasses—WAGO-class and CSR-class—based on their Argonaute binding partners and downstream regulatory functions.

Despite their biochemical similarities, WAGO-class and CSR-class 22G-RNAs are selectively bound by different Argonaute proteins. WAGO-class small RNAs are bound by WAGO-1, PPW-1, and PPW-2 and participate in germline gene silencing, while CSR-class small RNAs are bound by CSR-1 and WAGO-4 and contribute to genome surveillance and transgenerational inheritance respectively. How Argonaute proteins distinguish between these small RNA classes despite their near-identical biochemical properties remains unclear.

To investigate this question, I focused on five Argonaute proteins expressed in the *C. elegans* germline and localized to germ granules: CSR-1, WAGO-1, WAGO-4, PPW-1, and PPW-2. I found that small RNA binding is essential for maintaining proper Argonaute localization within germ granules. Furthermore, we observe that WAGO-1 exhibits strict binding specificity for WAGO-class small RNAs, while WAGO-4 demonstrates greater flexibility, switching to WAGO-class small RNAs when its preferred CSR-class small RNAs are unavailable. This suggests that WAGO-1 and WAGO-4 employ distinct mechanisms to achieve small RNA specificity.

Our findings suggest that additional regulatory factors contribute to Argonaute specificity. We identify a chaperone protein that could be a key regulator of WAGO-1 binding specificity. Ongoing work aims to further dissect how this chaperone and other protein cofactors contribute to Argonaute-small RNA binding preferences and gene silencing outcomes.

538C Sensory neurons work collaboratively to regulate peroxide resistance

Naadia Z Rashid, Daniel Shaw, Meha Macwan, Chloe Zenker, Javier Apfeld Biology, Northeastern University

One of the most common lethal threats that nematodes encounter is hydrogen peroxide, which is produced by a wide variety of microorganisms and by photochemical reactions. Preventing and repairing the damage induced by peroxides and other reactive oxygen species is critical for survival. Our lab's previous work revealed that *C. elegans* integrates diverse sensory inputs to determine when to induce peroxide defenses in target tissues. At least ten classes of sensory neurons can either lower or increase peroxide resistance. Here, we set out to explore whether these neurons function independently or work together to regulate peroxide resistance.

Using genetic approaches, we built a collection of strains where one or two classes of sensory neurons were genetically ablated via neuron-class specific expression of caspase. We focused on four classes of sensory neurons: ASK, ASG, AWC, and ASJ. We performed tert-Butyl hydroperoxide survival assays at 20°C. Our results revealed context-dependent effects. Specifically, (a) ASG and AWC neurons acted in a non-additive manner: AWC ablation increased survival by 8% when ASG neurons were intact but had no additional effect in ASG-ablated worms, which lived 48% longer than controls. (b) ASJ was required for ASG or AWC to influence survival: neither AWC nor ASG ablation extended lifespan in ASJ-ablated worms, where ASJ ablation alone reduced survival. (c) ASJ and ASK acted independently of one another: the ASK ablation decreased survival similarly in both ASJ-ablated and intact worms. In the future, we plan to compare the effects on defense gene expression of single and double neuron-ablated worms.

539C Molecular characterization of an *nhr-25* DNA Binding Domain mutant

John Ready, Abraham Hernández Mujica, Belle Ange Itetere, Kimberley T Muchenje, Deborah M Thurtle-Schmidt
Biology, Davidson College

Mutations in transcription factors (TF), specifically in the DNA binding domain, are common disease variants, impairing TF function. Yet how individual mutations in the TF protein sequence affect DNA binding recognition is poorly understood especially in the *in vivo*, cellular context. NHR-25 is an excellent model to determine the impact of point mutations on TF function as mutations in the DNA binding domain of the human ortholog show a range of phenotypes in humans including XY sex reversal and XX ovarian failure. The well-studied *nhr-25(ku217)* mutant, which has a single leucine to phenylalanine mutation in the DNA binding domain, exhibits phenotypes consistent with *nhr-25* defects, but must be partly functional as the mutant can be maintained as a homozygote, whereas deletion of *nhr-25* is lethal. Additionally, Chen et al. showed that in gel shift assays this mutation abolishes NHR-25-DNA binding *in vitro* to the presumed response element (2004). To determine the impact of this mutation on transcription, we performed RNA-seq in L3 worms in *nhr-25(ku217)*, *nhr-25(RNAi)*, and wild-type worms. Differential expression revealed a core set of genes showing altered expression in both the *nhr-25(ku217)* mutant and those worms in which *nhr-25* is knocked down by RNAi. Additionally, a subset of genes also showed differential regulation, primarily upregulation, specific to the *nhr-25(ku217)* mutant. This result suggests that the point mutant still regulates a subset of genes and possibly exhibits neomorphic activity, binding to a novel response element. To determine binding of the point mutant, we are performing CUT&RUN on the endogenously tagged *nhr-25(ku217)* allele. Additionally, the *nhr-25(ku217)* mutant is temperature sensitive showing more severe egg-laying defects at 25˚C as compared to 15˚C. This difference suggests that the mutant is more functional at lower temperatures. To identify genes differentially responsive to *nhr-25* activity we are profiling the *nhr-25(ku217)* transcriptome at higher and lower temperatures in L3 worms. By integrating transcriptome and binding profiles from a DBD point mutant, we can characterize how a single nucleotide polymorphism alters TF function.

540C Exploring the *cis*-regulation of a developmental switch gene in *Pristionchus*

Shelley J. Reich[1], Samantha Nestel[2], Michael Werner[21]School of Biological Sciences, University of Utah, [2]University of Utah

The nematode *Pristionchus pacificus* has emerged as a model system for studying developmental plasticity. These worms can develop either a bacterivore mouth with a single tooth or an omnivore mouth with two teeth that enables predation on other nematodes in addition to feeding on microbes. Mouth-form plasticity is influenced by environmental conditions during larval development and genetic background. Genetic analysis has identified multiple genes which affect mouth-form. Here, we show that environmental conditions induce differential expression of two switch genes, *eud-1* and *seud-1*, during the critical window for mouth-form plasticity. We calculated promoter nucleotide diversity across 104 natural isolates of *P. pacificus*, and found low promoter nucleotide diversity for *eud-1* compared to the genome-wide distribution. Previous work has demonstrated that copy number variation of a transcription factor binding site 3 kb upstream of *eud-1*and within its first intron contribute to mouth-form plasticity. We interrogated the 1.5 kb immediately upstream of *eud-1* and the first 500 bp after the start codon for additional *cis*-regulatory elements. We first aligned the *eud-1* promoter from five *Pristionchus* species and identified two highly conserved transcription factor binding sites. CRISPR mutagenesis of these sites did not affect mouth-form. We also identified additional intraspecies variants including a 235 bp deletion in the *eud-1* promoter and a 19 bp deletion in the first exon which introduces a premature stop codon. Surprisingly, we found an alternative start codon in the second exon with an open reading frame. Generation of the 235 bp deletion in the reference line PS312 showed no effect on mouth-form. In summary, the *eud-1* promoter exhibits evidence of both intra- and interspecies conservation, however the *cis*-regulation of *eud-1* appears robust to mutation. We are now systematically making large deletions to determine whether portions of the proximal promoter are important for *eud-1* regulation. Identification of specific regulatory elements in the *eud-1* promoter would pave the way for identifying transcription factors that regulate this key developmental switch gene.

541C Trivially-easy targeted integration of extrachromosomal arrays using PhiC31

Matthew S Rich[1], Adam J Hefel[1], Erik M Jorgensen[1,21]School of Biological Sciences, University of Utah, [2]Howard Hughes Medical Institute

Extrachromosomal arrays are unique chromosome-like structures created from DNA injected into the *C. elegans* germline. Arrays are easy to create and allow for high expression of multiple transgenes. They are, however, unstable unless integrated into a chromosome. Current methods for integration, such as X-rays and CRISPR, damage DNA and are low-efficiency. Here, we show that the viral integrase PhiC31, which mediates a non-mutagenic recombination between short attB and attP sequences, can be used for extremely efficient targeted array integration. In this method, we inject DNA containing a transgene, a selectable marker (such as a drug resistance cassette), and attP sites into a strain that (1) has an attB site in its genome, and (2) expresses PhiC31 in its germline. F1 extrachromosomal arrays are cloned, grown for multiple generations with selection, and then screened for homozygous array integrations. We recover integrations with nearly 100% efficiency from lines that transmit an array to the F2 generation. This process is simple, requires very little extra hands-on time compared to screening for extrachromosomal arrays, and allows us to screen arrays for function after they are stably integrated. We envision this method should drive a shift for *C. elegans* researchers from using unstable extrachromosomal arrays to direct targeted genomic integration with PhiC31.

542C Investigating Nuclear RNAi in Small RNA Biogenesis and Transcriptional Termination

Eva Richard, Rene Ketting Institute of Molecular Biology

RNA interference (RNAi) is a gene regulation mechanism mediated by Argonaute proteins (AGOs), which associate with small RNAs (sRNAs) to target complementary transcripts. In *C. elegans*, nuclear RNAi involves secondary AGOs (HRDE-1 and NRDE-3). These AGOs, loaded with sRNAs, translocate into the nucleus and recruit NRDE factors (e.g., NRDE-2) to repress gene expression by inhibiting RNA polymerase II elongation and promoting repressive chromatin modifications.

Recently, the RNA helicase MTR-4 has been identified as a key nuclear RNAi component, which associates with NRDE-2 at nascent transcripts. The conserved NRDE-2/MTR-4 complex plays a crucial role in nuclear RNAi, but its precise function remains unclear. While MTR-4 is known to facilitate RNA degradation via the exosome, emerging evidence suggests that NRDE-2-bound MTR-4 may protect nascent RNAs from degradation in mammals. We aim to investigate the function of NRDE-2/MTR-4 in *C.elegans*, hypothesizing that if this function is conserved in worms, it could help preserve transcripts for processing, sRNA amplification, and reinforced gene silencing.

Expanding on sRNA processing and export, we also aim to explore a well-characterized complex that plays a major role in transcript fate: the cap-binding complex (CBC). Composed of NCBP-1, NCBP-2, and the key factor SRRT-1 (the *C.elegans* ortholog of ARS2), the CBC regulates transcript processing, termination, RNA export, and degradation. Its interaction partners ultimately determine a transcript's fate. However, these interactions remain largely unexplored in *C. elegans*.

543C Transgenerational defects in chemotaxis behavior caused by the heritable accumulation of histone modifications across generations

Mackenzie S Roberson[1], Monica Reeves[2], Liyang Sun[2], Keila Garcia-Parda[3], Jaely Z Chavez[3], Karen Schmeichel[3], David J Katz[21]Biology, Emory University, [2]Emory University, [3]Oglethorpe University

Previously, we showed that mutations in *wdr-5*, an essential subunit of the COMPASS complex required for depositing H3K4 methylation, and *jhdm-1,* a putative H3K9me2 demethylase, lead to the heritable accumulation of H3K9me2 and a transgenerational increase in longevity. Similarly, we find that loss of *spr-2*, a histone acetyltransferase inhibitor, leads to the heritable accumulation of histone acetylation and transgenerational sterility (germline mortality). To understand whether the accumulation of H3K9me2 and histone acetylation affects behavior, we performed chemotaxis in *wdr-5, jhdm-1* and *spr-2* mutants. We find that all three mutants have chemotaxis behavior defects. In *wdr-5* and *jhdm-1* mutants, the chemotaxis defect becomes more severe across generations. Preliminary data suggest that this may also occur in *spr-2* mutants. Taken together, these findings suggest that heritable histone modifications can result in transgenerational changes to behavior. Interestingly, human patients with mutations in the orthologs of WDR-5 and SPR-2 have neurodevelopmental disorders with altered behavior. Thus, by investigating how heritable histone modifications cause transgenerational chemotaxis defects, we hope to gain insight into how inappropriately inherited histone modifications alter behavior.

544C Regulation of circuit formation by circuit-organizer transcription factors

Jonathan D Rumley[1], Oliver Hobert[1,21]Biological Sciences, Columbia University, [2]Howard Hughes Medical Institute

The Hobert lab has uncovered a class of terminal selector transcription factors that regulate the identities and, potentially, the connections of all the neurons in interconnected circuits in the *C. elegans* nervous system. Loss of one of these circuit-organizer transcription factors, UNC-42, results in a reduction in synapses of neurons that normally express *unc-42*. We seek to determine the mechanism by which circuit-organizer transcription factors carry out their functions in regulating the formation and/or maintenance of synapses between neurons that express them. We have hypothesized that circuit-organizer transcription factors regulate synapse formation and maintenance through regulation of *unc-6* expression, which has been shown to be required for synapse formation or maintenance in particular cases. We have deleted portions of the *unc-6 cis*-regulatory region (upstream and introns) and have identified regions required for expression of *unc-6* in specific cells. In particular, we have identified predicted UNC-42 binding sites in intron 7 which are required for expression of *unc-6* in the command interneurons AVA, AVB, AVE, and AVD. We have observed preliminarily that worms in which a region including these sites is deleted exhibit a decrease in the proportion of their movement spent reversing, suggesting a defect in the function of the command interneurons, which regulate the decision of forward versus reverse movement. We, however, as of yet, have not observed a defect in synapses of command interneurons in *unc-6* mutants. Thus, we are considering the hypothesis that UNC-42 regulates the formation and maintenance of synapses in the command interneurons through regulation of the immunoglobulin cell adhesion molecules (IgCAMs) *rig-3*, *rig-6*, *rig-5*, and *rig-1*, each of which is expressed in at least one of the command interneurons. These proteins mediate cell-cell adhesions, and most are localized to the synapse. To determine if IgCAMs regulate the synapses of command interneurons, I am deleting them using CRISPR-Cas9. I will examine the effects of their loss on synapses of the command interneurons using GRASP. If any IgCAMs are required for synapse formation and/or maintenance in the command interneurons, I will determine its expression pattern by inserting a co-expressed GFP reporter at its locus and determine the requirement of UNC-42 for its expression in command interneurons by deleting predicted binding sites from its *cis*-regulatory region.

545C The knockdown of lipase *atgl-1* dysregulates autophagy in *C. elegans*

Sara Sampson, Joslyn Mills Biology, Bridgewater State University

Autophagy is the crucial process of breaking down and recycling cellular components, and it is conserved in organisms ranging from microscopic nematodes to humans. The dysregulation of autophagy has been implicated in aging and numerous diseases, importantly in protein aggregation-associated diseases such as Huntington's and Alzehimer's diseases. A reverse genetic screen of RNAi against 80 genes in an autophagy model of *C. elegans* identified adipose triglyceride lipase 1 (*atgl-1*) as a modulator of autophagy as seen by the increase of GFP fluorescence when *atgl-1* is knocked down. qPCR data revealed that *atgl-1* knockdown downregulates the gene expression of selected autophagic markers, suggesting *atgl-1*i causes a blockage of autophagy at the midpoint of the process. To identify at which point *atgl-1*i is disrupting this pathway, double-knockdown of selected autophagy genes and *atgl-1* was performed. Behavioral and molecular assays were completed in order to determine the role of *atgl-1* in regulating autophagy and energy allotment, including heat stress and fecundity assays to determine if the stress response or reproduction, respectively, were affected. This investigation provides a basis for further study of *atgl-1*'s impact on autophagy.

546C Nonsense-Mediated Decay Regulates SPD-3, a Mitochondrial-Localized Protein Essential for Cell Division

Samantha H Schaffner, David I Taylor, Adrianna M Strozak, Charlie C Edwards, Maulik R Patel Biological Sciences, Vanderbilt University

Caenorhabditis elegans are often used as a model for the study of highly conserved processes, however, they are also a powerful system for exploring newly evolved processes that can offer unique biological insight. The newly evolved nature and peculiar localization of the SPD-3 protein suggests it might be involved in a unique role. *Spd-3* is essential for early embryonic viability, as mutants have a sterile phenotype due to disruption of meiotic and mitotic spindle regulation which results in abnormal polar body extrusion, chromosome segregation, and spindle alignment. However, despite these cell division phenotypes, SPD-3 localizes to mitochondria, an uncharacteristic localization for a protein so specifically involved in cell division. Little is known about how SPD-3 functions in cell division with this mitochondrial localization. To assess the regulation of *spd-3,* I conducted a forward genetic screen on a *spd-3* temperature-sensitive mutant and assessed for a rescue in embryonic viability. From this screen, mutations in nonsense-mediated decay, the process in which mRNAs that contain premature termination codons are degraded, were found to rescue the embryonic viability in temperature-sensitive *spd-3.* I will present data on our assessment of *spd-3* transcripts using digital droplet PCR to determine if nonsense-mediated decay is directly regulating *spd-3* at the mRNA level. I will also present data from my biochemical efforts to identify interacting partners of SPD-3. Altogether, this work examines the regulation of a novel cell division mechanism and provides insights into the diverse evolution of basic cellular processes.

547C CGC1, a new reference genome for *Caenorhabditis elegans*

Erich M. Schwarz[1], Kazuki Ichikawa[2], Massa J. Shoura[3], Karen L. Artiles[3], Dae-Eun Jeong[3], Chie Owa[2], Haruka Kobayashi[2], Yoshihiko Suzuki[2], Manami Kanamori[4], Yu Toyoshima[4], Yuichi Iino[4], Ann E. Rougvie[5], Lamia Wahba[6], Andrew Z. Fire[7], Shinichi Morishita[21]Dept. of Molecular Biology and Genetics, Cornell University, [2]Dept. of Computational Biology and Medical Sciences, University of Tokyo, [3]Dept. of Pathology, Stanford University, [4]Dept. of Biological Sciences, University of Tokyo, [5]Dept. of Genetics, Cell Biology, and Development, University of Minnesota, [6]Lab. of Non-Canonical Modes of Inheritance, Rockefeller University, [7]Depts. of Pathology and Genetics, Stanford University

The original 100.3 Mb reference genome for *Caenorhabditis elegans*, generated from the wild-type laboratory strain N2, has been crucial for analysis of *C. elegans* since 1998 and has been considered complete since 2005. Unexpectedly, this long-standing reference was shown to be incomplete in 2019 by a genome assembly from the N2-derived strain VC2010. Moreover, genetically divergent versions of N2 have arisen over decades of research and hindered reproducibility of *C. elegans* genetics and genomics. Here we provide a 106.4 Mb gap-free, telomere-to-telomere genome assembly of *C. elegans*, generated from CGC1, an isogenic derivative of the N2 strain. We used improved long-read sequencing and manual assembly of 43 recalcitrant genomic regions to overcome deficiencies of prior N2 and VC2010 assemblies, and to assemble tandem repeat loci including a 772-kb sequence for the 45S rRNA genes. While many differences from earlier assemblies came from repeat regions, unique additions to the genome were also found. Of 19,972 protein-coding genes in the N2 assembly, 19,790 (99.1%) encode products that are unchanged in the CGC1 assembly. The CGC1 assembly also may encode 183 new protein-coding and 163 new ncRNA genes. CGC1 thus provides both a completely defined reference genome and corresponding isogenic wild-type strain for *C. elegans*, allowing unique opportunities for model and systems biology.

548C Investigating Germline-Specific MicroRNA Regulation

Mathieu Segonds, Vaishnav Vasudevan, Martin SimardCHU de Québec-Université Laval Research Center

MicroRNAs (miRNAs), first identified in the nematode *Caenorhabditis elegans*, are now recognized as crucial regulators of various biological processes, including cell division and differentiation. Due to their critical role in maintaining cell homeostasis, miRNA dysregulation has been linked to several human diseases, including cancer. Mechanistically, upon association with Argonaute proteins, these small non-coding RNAs assemble into an effector complex known as the miRNA-induced silencing complex (miRISC). This complex primarily interacts with target mRNAs in their 3' untranslated region through sequence complementarity, thereby modulating gene expression. In cellulo studies have revealed several mechanisms by which miRNAs influence gene expression, and our research aims to better understand these processes in *C. elegans*.

In *C. elegans*, our previous studies have revealed tissue-specific gene regulatory mechanisms driven by distinct molecular compositions of miRISC, particularly in germline and somatic tissues. Given that miRNAs exert tissue-specific regulatory functions through different miRISC compositions, we hypothesized that unidentified genetic factors contribute to this specificity.

To identify the genetic factors involved, we have developed a novel approach using a dual reporter (GFP & RFP) strain of *C. elegans* sensitive to disruptions in the miRNA pathway. This approach allows live fluorescence imaging in both somatic and germline tissues, providing a dynamic view of miRNA regulation in real time.

Using a forward genetic screen, we chemically mutagenized 500,000 genomes and screened approximately 24,000 of them to identify viable candidate worms. From this, we isolated 15 candidates exhibiting tissue-specific miRNA regulation defects. These included ten germline-deficient miRNA regulatory mutants (GEDI) and five somatically deficient mutants (SODI). Preliminary characterization of the GEDI mutants revealed significant germline developmental defects.

Based on these observations, we are currently conducting further phenotypic and molecular characterizations of the various GEDI mutants, profiling them and identifying the causative mutations. At this meeting, I will present our latest findings on the GEDI mutants, which include the identification of candidate genes involved in tissue-specific miRNA regulation. Additionally, we will highlight how our dual reporter system has facilitated precise *in vivo* monitoring of miRNA pathway disruptions in *C. elegans*.

549C Regulation of gametic gene expression across development via RNAi-to-RNAi cascade in *C. elegans*.

Trilotma Sen[1], Alicia Rogers[21]Biology, The University of Texas at Arlington, [2]Biology, The University of Texas at Arlington

Eukaryotes use RNA interference (RNAi) to ensure precise and coordinated regulation of gene expression throughout development. In hermaphroditic *C. elegans*, RNAi regulates gene expression to temporally coordinate spermatogenesis and oogenesis in the germline. However, the mechanism of how this temporal gametic gene regulation is controlled, especially during stress, remains understudied. Our central hypothesis dictates that RNAi pathways employ autoregulation and feedback mechanisms to coordinate small RNA pathway functions essential for the regulation of spermatogenesis genes. We previously reported evidence of a feedback mechanism manifesting as a genetic switch operated via *Mutator*-dependent siRNAs to regulate the Argonautes' ALG-3 and ALG-4 expression in a temporal manner. This RNAi-to-RNAi cascade fine-tunes ALG-3/4 RNAi pathway to restrict sperm gene expression at L4 stage, particularly during heat stress. To dissect this temperature dependent regulatory architecture of controlling *alg-3* and *alg-4*, we propose that *Mutator* complex-dependent 22G-RNAs target discrete regions in the first and second exons of *alg-3* and *alg-4,* in concert with autoregulatory feedback from ALG-3/4-bound 26G-RNAs. Here, we are using complementary approaches to examine the feedback motif that coordinates *alg-3* and *alg-4* gene expression. We approach to bypass the influence of *Mutator* focus on *alg-3* and *alg-4* by leveraging a guide RNA-based feeding assay to overexpress *alg-3* and *alg-4*. We will also use directed mutagenesis to change *alg-3* and *alg-4* loci such that they are no longer targeted by small RNAs. Together these studies will provide a foundational map of molecular interactions in RNAi functions, and how small RNA pathways minimize the stochastic fluctuations in gene expression ensuring proper coordination of the gametic genome.

550C SLRanger, a pipeline for the de novo identification of splicing leader (SL) sequences using full-length RNA sequencing data.

Yanwen Shao, Zhihao Guo, Runsheng Li City University of Hong Kong

We present SLRanger, a pipeline for the de novo identification of splicing leader (SL) sequences using full-length RNA sequencing data. Our approach first maps known SL sequences onto the transcriptome and then examines soft-clipping regions to identify additional SL candidates. By employing a scoring system based on the Smith-Waterman algorithm (SSW) —further refined with features specific to SL sequences—our method optimizes the detection of spliced leader events and outperforms traditional SSW mapping when compared against random mapping. This enhanced scoring system not only accurately distinguishes between random sequences and genuine spliced leader sequences but also differentiates SL1 from various SL2 variants. Validation using data from *Caenorhabditis* possessing splicing leader structures—including *C. elegans*, *C. briggsae*, and *C. nigoni*. Consequently, our tool enables the determination of full-length long RNA reads, which could provide a more comprehensive overview of splicing patterns. Besides, the detection of SL2 reads could help in expanding the operon structures and annotations in the used species.

551C Uncovering the transcriptome patterns of *Caenorhabditis briggsae* and *C. nigoni* at different stages using Nanopore sequencing

Yanwen Shao, Yiwen Zhang, Runsheng Li City University of Hong Kong

Annotations on the transcriptome of most *Caenorhabditis* species were insufficient. Here, we want to decode the transcriptome of *C. briggsae* and *C. nigoni* using nanopore direct RNA sequencing technology, which could largely produce the full length of RNA. We have sequenced three different stages of the two species, including embryo, larva stage one, and young adults. Through the comparison between different stages' transcriptomes, we gave more specific explanations of gene functions and annotations. Also, through comparing cDNA full-length sequencing of F1 hybrids, we can also know more about differential expression between hybrids and parent species in the isoform level. This study also provide resource value on the HI in *C. briggsae* and *C. nigoni.*

552C Neural regulation of insulin-like hormones in brain-body communication

Eva Sheardown, Jane Yan To Ling, Fursham Hamid, QueeLim Ch'ng Centre for Developmental Neurobiology, King's College London

Neurotransmitters and hormones are both found in the brain and control physiological processes in the body. How these two systems interact is not well understood. Discovering the circuitry linking the two will give insight into neural control of physiological functions. In addition, the pattern of these connections can reveal their decision-making functions. Our main hypothesis is that neurotransmitters modulate expression of hormones in the nervous system, and this pattern of regulatory connections produces information processing functions that link neural activity to bodily physiology.

To understand the genetic mechanism underlying neuroendocrine control of growth and development, we investigate the regulation of insulin-like peptides (ILPs) in sensory neurons. These ILPs regulate a developmental switch between growth and a specialised larval arrest called dauer 1. The key ILPs regulating this developmental decision are *ins-4*, *ins-6*, and *daf-28* 2. These ILPs mutually compensate for the loss of individual signals, thereby producing redundancy, where loss of all 3 ILPs is required for a high rate of dauer entry 3. Although much is known about these ILPs, the neural signals regulating them are less well understood.

To investigate this, we have tested loss-of-function mutants in genes in neurotransmitter pathways for altered rates of dauer arrest. We implicated several neurotransmitters in regulation of dauer entry. Future experiments using Hybridisation Chain Reaction 3.0 to visualise single transcripts and cell-specific rescue will identify how these neurotransmitters regulate ILPs to uncover the pattern of regulatory connections linking neural activity to body physiology.

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553C Spatial Interactions of DNA Break Regions During Nematode Programmed DNA Elimination

James Simmons, Tianchun Xue, Rachel McCord, Jianbin Wang BCMB, The University of Tennessee

Genome integrity is critical for cellular functions, and chromosomal breaks can have catastrophic consequences. One notable exception is Programmed DNA Elimination (PDE). In one form of PDE that occurs in many nematodes, DNA double-strand breaks (DSBs) are induced at specific chromosomal regions to fragment DNA, leading to subsequent loss of chromosomal pieces. Studies in these nematodes have significantly advanced our understanding of PDE, demonstrating that it can 1) silence germline-specific genes by removing them from the somatic genome; 2) remodel chromosome ends through the removal of germline subtelomeric sequences and telomeres and synthesis of new somatic telomeres; and 3) change karyotype in some species. A key question in the mechanism of PDE is the recognition of the break sites. We found in the free-living nematode *Oscheius tipulae*, a conserved motif is used to generate DNA breaks. In contrast, in the human and pig parasitic nematode *Ascaris*, no such motifs are present, raising questions about how break sites are recognized in *Ascaris*. We hypothesize that 3D interactions contribute to break site recognition prior to PDE. Using Hi-C, we found the break sites engage in spatial interactions before PDE in *Ascaris*, suggesting that physical interactions between these sites may contribute to the PDE process. Notably, these interactions are established in the germlines of both males and females, suggesting the 3D interactions may be an inherent feature of the to-be-eliminated sequences. They could serve a germline-specific function such as pairing during meiosis. Thus, PDE in *Ascaris* may provide a means to disrupt a germline function that is no longer needed in the somatic cells. Interestingly, breaks in the middle of 11 *Ascaris* germline chromosomes also lead to karyotype changes in the somatic cells. Following PDE, many genes originally located in the central regions of a germline chromosome become terminal in the somatic cells. This repositioning may alter the 3D genome organization and chromatin state of these regions, providing an additional mechanism of gene expression regulation. Collectively, our findings contribute to a model that may explain how sequence-independent break sites are recognized for *Ascaris* PDE. Our studies also provide insights into a relationship between 3D genome conformation and karyotype changes.

554C Investigating how the transgenerational accumulation of repressive H3K9me2 affects health and lifespan

Marybeth Slack, Thomas Wolfe, Scott P Roques, Jaime C Croft, Teresa W Lee University of Massachusetts Lowell

Generational studies suggest that the experiences of ancestors can affect the health of their descendants, in part by altering how genomes are packaged as chromatin. We have developed a novel *C. elegans* model for transgenerational epigenetic inheritance to examine how heritable chromatin landscapes affect gene regulation and complex traits like lifespan. Mutations in WDR-5 and other components of the COMPASS H3K4 methyltransferase complex extend lifespan and enable its inheritance. Previously, we have shown that *wdr-5* mutant longevity is itself a transgenerational trait that corresponds with a global enrichment of the heterochromatin factor H3K9me2 over twenty generations. Additionally, the transgenerational acquisition of *wdr-5* mutant longevity requires the H3K9me2 methyltransferase MET-2 and is recapitulated by removal of the putative H3K9me2 demethylase JHDM-1. In both *wdr-5* mutants and *jhdm-1* mutants, longevity is associated with a corresponding transgenerational increase of the repressive modification H3K9me2, particularly over genes expressed in the germline. Taken together, these results suggest that repressive chromatin landscapes in the germline enables the transgenerational establishment and inheritance of longevity. Intriguingly, we find that although both mutants eventually attain longevity, they do so with different generational dynamics and striking differences in health. We are currently examining gene expression and health metrics to address whether healthspan is proportionally extended in long-lived mutant populations. This work will identify how the inheritance of repressive chromatin landscapes affects genetic pathways that control the complex relationship between health and lifespan.

555C Single-Nucleus Neuronal Transcriptional Profiling of *C. elegans* Reveals Regulators of Cognitive Aging and Sexual Dimorphism

Katherine Morillo[1], Jonathan St. Ange[2], Yifei Weng[1], Rachel Kaletsky[1], Coleen Murphy[1]1Molecular Biology, Princeton University, [2]Princeton University

Sexual differentiation of the nervous system causes differences in neuroanatomy, synaptic connectivity, and physiology. These sexually-dimorphic phenotypes ultimately translate into profound behavioral differences. *C. elegans'* two sexes, XO males and XX hermaphrodites, demonstrate differences in neurobiology and behavior. However, the neuron class and sex-specific transcriptomic differences, particularly at the single-neuron level, that cause such phenotypic divergence remains understudied. Here, using single-nucleus RNA sequencing, we assessed and compared adult male and hermaphrodite *C. elegans* neuronal transcriptomes, identifying sex-specific neurons, including previously-unannotated male neurons. Sex-shared neurons displayed large expression differences, with some neuron classes clustering as distinct neurons between the sexes. Males express 100 male-specific GPCRs, largely limited to a subset of neurons. We identified the most highly-divergent neurons between the sexes, and functionally characterized a sex-shared target, *vhp-1,* in male-specific pheromone chemotaxis. Our data provide a resource for discovering nervous-system-wide sex transcriptomic differences and the molecular basis of sex-specific behaviors.

556C Transposons nucleate condensation of HSF-1 during heat shock

Natalia Stec, Tugba Duran, Barbara Hanulak, Adam Klosin Laboratory of Spatial Epigenetics, Nencki Institute of Experimental Biology, Polish Academy of Sciences

HSF-1 is an essential eukaryotic transcription factor that forms nuclear stress bodies (nSBs) in response to heat. The exact function of these structures is unknown. Through quantitative analysis of *C. elegans* embryos we found that during heat shock, HSF-1 condenses into four major nSBs, in addition to tens of minor ones. Surprisingly for a transcription factor, HSF-1 foci remain prominent even on packed chromatin throughout mitosis when transcription is severely halted. What is more, nSBs do not enrich RNA Polymerase II and are not disrupted by its depletion. The majority of the HSF-1 binding sites in the *C. elegans* genome reside within Helitrons, a class of DNA transposons. Using DNA FISH, we show that major nSBs strongly co-localize with Helitron repeats. Analysis of the genome sequence identified several regions with extremely high local enrichment of HSF-1 binding sites. Using CRISPR-Cas9 editing we deleted one of these regions, which resulted in the disappearance of two out of four major nSBs. This suggests that transposable elements nucleate condensation of the majority of HSF-1 during heat shock. Using RNA-seq, reporter studies and phenotypic assays, we are currently in search of molecular phenotypes to help us understand the function of these Helitron associated nSBs in regulating the transcriptional response to heat shock.

557C Heritable histone acetylation across generations in SPR-2/INHAT mutants causes germline mortality

Liyang I Sun[1], Monica N Reeves[1], Keila Garcia-Parada[2], Karen Schmeichel[2], David J Katz[1]1Cell Biology, Emory University, [2]Biology, Oglethorpe University

Histone acetylation is an epigenetic marker associated with active transcription that can be used to help maintain cell type specific gene expression. SPR-2 (INHAT/SET in mammals) binds to chromatin and INhibits Histone AcetylTransferase binding to dampen the acquisition of histone acetylation. Since the acquisition of histone acetylation is a universally conserved mechanism to help maintain gene expression, why would certain cells need to dampen the acquisition of histone acetylation? During the maternal-to-zygotic transition, histone modifications get reprogrammed to prevent the inappropriate maintenance of germline expression in the embryo of the subsequent generation. We find that loss of SPR-2 results in the transgenerational accumulation of histone acetylation throughout the genome and sterility across generations (germline mortality). This suggests that SPR-2 dampens acquisition of germline histone acetylation to prevent high levels of histone acetylation which could overwhelm epigenetic reprogramming and be transmitted transgenerationally. These data provide the first evidence that histone acetylation can be stably inherited across generations. Additionally, we show that the accumulation of histone acetylation in *spr-2* mutants leads to developmental delay and abnormal chemotaxis behavior, phenotypes that are observed in the corresponding human patients. Based on this overlap in phenotypes, we are using *spr-2* mutant *C. elegans* to understand how inappropriately inherited histone acetylation gives rise to neurodevelopmental defects.

558C Bone Morphogenetic Protein Signaling at a Novel Regulatory Nexus of Lipid Trafficking and Metabolism in *C. elegans*

Allen Sun[1], Katerina Yamamoto[2], Cathy Savage-Dunn[21]Biochemistry, City University New York Queens College, [2]Biology, City University New York Queens College

Bone Morphogenic Protein (BMP) ligands of the greater Transforming Growth Factor β (TGF-β) family of signaling peptides are well conserved across the metazoan animal kingdom and recognized for their functional roles in organismal development and homeostasis. The *C. elegans* DBL-1 homolog of BMP2/4 known for controlling body size, development, and differentiation also coordinates the regulation of intestinal fat deposits. Previous transcriptional analyses in our lab identified several target genes of the DBL-1 signaling pathway involved in lipid metabolism where studies show the loss or overexpression of the ligand result in the reduction of fat accumulation. Two of these target genes are the lipid binding protein (LBP) genes *lbp-5* and *lbp-8* that are related to vertebrate fatty acid binding proteins (FABPs). In vertebrates, the regulation of lipid metabolism is controlled by FABPs, fatty acid ligands, and their cognate downstream nuclear hormone receptors (NHRs). Evidence to support nucleoplasmic shuttling of fatty acid ligands and the collisional interactions between LBPs and NHRs have focused on coregulatory roles of LBP-5 and NHR-49 modulating their respective expression of target genes in fat metabolism. However, there is a paucity of information as to how BMP signaling can influence fatty acid ligands trafficking from lipid reservoirs to differentially regulate NHR transcriptional responses. We hypothesize BMP/DBL-1 signaling mediates the expression of LBPs to deliver fatty acid ligands to NHRs thereby regulating the targeted expression of genes involved in lipid metabolism. To examine these mechanisms, I will first analyze double mutants of *lbp* and *dbl-1* signaling; second, future studies will identify the relevant NHRs (e.g. NHR-49); third, I will identify which specific fatty acid ligands differentially contribute to BMP signaling mediating lipid metabolism. Taken together, these findings will ultimately add new insights for novel treatment modalities in diabetes and other metabolic disorders.

559C Dissecting the role of the GHKL ATPase MORC-1 in germline gene regulation

Tian Tan[1], Jessica A Kirshner[1], Amelia F Alessi[2], Colette Picard[3], Natasha E Weiser[4], Steven E Jacobsen[3], John K Kim-[11]Johns Hopkins University, [2]Biology, Johns Hopkins University, [3]University of California, Los Angeles, [4]Stanford University

Microrchidia (MORC) proteins are highly conserved GHKL ATPases that function as transcriptional repressors across species, playing roles in heterochromatin compaction in *Arabidopsis* and transposon silencing in mice. In *C. elegans*, MORC-1 is the sole homolog in this family and is closely related to human MORC2. MORC-1 is required for transgene silencing and the nuclear RNAi pathway. Recent study in our lab (Kirshner et al., 2024) demonstrated that MORC-1 acts downstream of the CSR-1 Argonaute to license germline gene expression, likely by altering the chromatin states. *In vitro* studies have shown that MORC-1 dimerizes and compacts DNA in a sequence-independent manner through a loop-trapping mechanism (Kim et al., 2019). However, the precise mechanism by which MORC-1 regulates germline gene expression *in vivo* remains unclear. To address this, I propose: 1) structure-function analysis of MORC-1 through the generation of mutant strains targeting conserved domains to investigate its role in germline gene regulation, and 2) germline-specific mass spectrometry analysis of immunopurified MORC-1 to identify MORC-1 interactors. Together, these experiments will provide mechanistic insights into MORC-1-mediated gene silencing in the germline, potentially revealing conserved mechanisms of epigenetic regulation.

560C Evidence for *Mutator* component-independent piRNA silencing

Wendy Tan, Craig Mello UMass Chan Medical School

Despite deep conservation of the piRNA pathway components and their well-established importance in fertility and genome defense, piRNA pathway silencing strategies diverge drastically across the animal kingdom. In the *Caenorhabditis elegans* germline, the PIWI homologue PRG-1 is thought to recruit the *Mutator* complex to piRNA targets to trigger mass production of 22G secondary siRNAs that associate with worm-specific WAGO Argonautes to carry out target silencing. However, germline transcriptomic analyses of *prg-1* and *Mutator* component *rde-3* or *mut-16* mutants reveal that roughly half of genes upregulated in *prg-1* animals are unaffected by *Mutator* complex loss. Therefore, we propose that PRG-1 can directly silence some piRNA targets using an as-yet undescribed *Mutator*-independent mechanism. We demonstrate that a novel piRNA-sensitive transgene reporter is targeted by *Mutator*-dependent and -independent piRNA silencing mechanisms. Unlike the canonical *C. elegans* piRNA pathway, *Mutator*-independent piRNA silencing does not trigger mass small RNA generation. Surprisingly, *Mutator*-independent piRNA silencing does not require PRG-1 catalytic activity and persists transgenerationally for a few generations after *prg-1*(+) activity is removed. This putative pathway requires *nrde-2*, a conserved protein first characterized as a nuclear silencing factor, but not other known WAGO nuclear silencing components such as *hrde-1* and *nrde-3.* Furthermore, we have identified putative endogenous targets of *Mutator*-independent piRNA silencing via gonadal RNA sequencing and RNA FISH. Collectively, these data demonstrate that PRG-1 can directly silence mRNAs through an epigenetic mechanism independent of the *Mutator* complex. Future work will focus on validating putative endogenous targets and identifying additional *cis* and *trans* components of the novel *Mutator*-independent piRNA silencing pathway.

561C Elucidating the role(s) of ubiquitin-mediated proteolysis factors on SKN-1 activity during pathogen infection of *C. elegans*

Larissa Tavizon, Carolaing Gabaldon, Melissa R. Cruz, Danielle A. Garsin Microbiology and Molecular Genetics, UTHealth Houston

During infection, the host mounts an immune response by producing reactive oxygen species (ROS) to defend against pathogens. However, excessive ROS production can be harmful to the host and cause adverse effects. In mammals, the Nrf family of transcriptional regulators mitigates oxidative stress by activating genes that protect against ROS-induced damage. Similarly, in *Caenorhabditis elegans*, a functional ortholog of the Nrf proteins known as SKN-1 is responsible for a comparable protective response during infection. Our current research focuses on investigating the regulatory mechanisms that control SKN-1 activity in the presence of pathogens, and whether these mechanisms involve distinct or overlapping factors. We discovered that the loss of *lin-23*, a component of the SCF complex associated with ubiquitin-mediated proteolysis, significantly reduces the expression of SKN-1 reporter genes *gst-4* and *gcs-1* in infected animals. Additionally, *lin-23* mutants exposed to pathogen exhibit increased susceptibility to infection, suggesting that *lin-23* acts as a positive regulator of SKN-1 during pathogen infection. Conversely, the WD repeat protein WDR-23 has been shown to facilitate SKN-1 degradation via the CUL4/DDB1 ubiquitin ligase complex, functioning as a negative regulator of SKN-1. Preliminary findings indicate that the loss of *lin-23* does not affect the constitutive activation of the SKN-1 reporter *gst-4p::GFP* in a *wdr-23* null background, suggesting that *wdr-23* is epistatic to *lin-23*. Moreover, the absence of *lin-23* does not alter PMK-1 phosphorylation levels, which is necessary for SKN-1 activation in response to oxidative stress, indicating that *lin-23* regulates SKN-1 activity downstream of the p38 MAPK signaling pathway. Together, our results indicate roles for ubiquitin ligase components WDR-23 and LIN-23 in regulating SKN-1 activity during infection. However, the precise mechanisms by which these factors influence SKN-1 remain unknown. Future studies will explore the hypothesis that LIN-23 promotes SKN-1 activity by targeting WDR-23 for degradation. A deeper understanding of SKN-1 regulation is critical for creating novel therapeutic approaches to combat drug-resistant infections.

562C Investigating the effect of transcription elongation rates on cell fate decisions in the developing *C. elegans* embryo

Gabriela Vida, Priya Sivaramakrishnan Children's Hospital of Philadelphia

The precise regulation of transcription and its machinery is essential to produce transcripts of specific genes at the necessary quantities in the proper cells at the right time. These precise levels of transcripts are in part what drive cell differentiation to be reproducible in developing embryos. Our lab and others have identified genes that are transcribed at high rates during development, however, the mechanisms that regulate high rate transcription and its effect on cell fate is unknown.

The *C. elegans* embryo is an essential model to study the effects of transcript number and dosage due to its invariant lineage, providing a way to carefully analyze the effects of transcript level changes and their consequences on cell fates. Previous work on transcription regulation primarily focuses on transcription initiation. Transcription elongation is also a highly regulated process, but its impact on cell fate decisions is still unclear. We hypothesize that regulation of these high rate genes during the developing embryo may depend on the regulation of elongation.

RNA Polymerase II (Pol II) is a multi-subunit enzyme complex that is essential for synthesizing mRNA from DNA. It is known that a missense mutation near the amanitin binding site in *ama-1*, the largest subunit of Pol II, transcribes at a slower rate. Preliminary evidence from our lab has shown that this slow Pol II mutant does ultimately cause transcripts to accumulate slower. We can therefore use this slow Pol II mutant to understand the developmental implications of slow transcription elongation. We will perform live imaging of embryos followed by lineage analysis to determine whether there are certain lineages that are more susceptible to fate changes due to slower elongation. Along with live imaging to detect overall cell fate changes, we can directly assay for transcript accumulation using single molecule FISH of known high-rate genes in the developing embryo. We can correlate the phenotypes we find with known elongation factor mutants like *cdk*-9. Additionally, we are assaying for how much functional Pol II is required for successful transcript accumulation by using a temperature sensitive *ama-1* mutant. We can ask how fine tuning the amount of functional Pol II can allow for successful embryogenesis.

Taken together, this work will shed new light on the role of transcription rates, specifically elongation, in promoting robust cell fate decisions required during rapid embryonic development.

563C A versatile site-directed gene trap strategy to manipulate gene activity and control gene expression in *Caenorhabditis elegans*

Haania Khan, Xinyu Huang, Vishnu Raj, Han Wang University of Wisconsin-Madison

The ability to manipulate gene activity and control transgene expression is essential to study gene function. While several genetic tools for modifying genes or controlling expression separately are available for *Caenorhabditis elegans*, there are no genetic approaches to generate mutations that simultaneously disrupt gene function and provide genetic access to the cells expressing the disrupted gene. To achieve this, we developed a versatile gene trap strategy based on cGAL, a GAL4-UAS bipartite expression system for *C. elegans*. We designed a cGAL gene trap cassette and used CRISPR/Cas9 to insert it into the target gene, creating a bicistronic operon that simultaneously expresses a truncated endogenous protein and the cGAL driver in the cells expressing the target gene. We demonstrate that our cGAL gene trap strategy robustly generated loss-of-function alleles. Combining the cGAL gene trap lines with different UAS effector strains allowed us to rescue the loss-of-function phenotype, observe the gene expression pattern, and manipulate cell activity spatiotemporally. We show that, by recombinase-mediated cassette exchange (RMCE) via microinjection or genetic crossing, the cGAL gene trap lines can be further engineered *in vivo* to easily swap cGAL with other bipartite expression systems' drivers, including QF/QF2, Tet-On/Tet-Off, and LexA, to generate new gene trap lines with different drivers at the same genomic locus. These drivers can be combined with their corresponding effectors for orthogonal transgenic control. Thus, our cGAL-based gene trap is versatile and represents a powerful genetic tool for gene function analysis in *C. elegans*, which will ultimately provide new insights into how genes in the genome control the biology of an organism.

564C Auxin-inducible protein degradation of the DRM complex reveals the dynamics of transcriptional repression of its direct gene targets in *Caenorhabditis elegans*

Emily Washeleski, Paul D Goetsch Biological Sciences, Michigan Technological University

Control over exit from the cell cycle is essential during development. The Dp, Retinoblastoma-like, E2F, and MuvB (DREAM) transcriptional repressor complex is a central regulator of cellular quiescence in mammals. In the conserved *Caenorhabditis elegans* DRM complex, we demonstrated that the MuvB subcomplex mediates DRM's repression of target genes. We are interested in understanding how the DRM complex functions as a transcriptional repressor. We hypothesize that the state of MuvB chromatin occupancy governs repression and derepression of target genes. However, in keeping with its vital role in development, mutational analyses of the DRM complex cannot overcome that viability requires residual complex activity and does not provide controlled temporal information of expression changes. Previous transcript analyses of DRM loss-of-function mutants revealed wide-spread indirect effects on gene expression. To overcome the limitations of mutational analyses, we integrated the Auxin-Inducible Degron (AID) system to degrade DRM complex proteins in *C. elegans.* Using CRISPR/Cas9-mediated genomic editing, we endogenously tagged two key DRM components, *lin-35* and *lin-54,* with a GFP-degron tag, and crossed each into a transgenic strain ubiquitously expressing *Arabidopsis thaliana* TIR1 E3 ubiquitin ligase. We observed degradation of DRM subunits within 2 hours post treatment with auxin compared to vehicle control. To assess the effects of DRM subunit degradation on DRM target gene regulation, we performed RNA-seq from auxin treated L1 larvae at 6- or 24-hour timepoints compared to vehicle control. We also performed RNA-seq from *lin-35(n745)* and *lin-54(n2231)* L1s compared to N2 L1s. We observed more precise upregulation of DRM target genes following degradation of LIN-35 or LIN-54 with limited indirect effects, as compared to mutational analysis. To further utilize the AID system to study DRM transcriptional dynamics, we performed single-cell RNA-seq from L1 larvae of the LIN-54 degron strain auxin treated for 24 hours, against vehicle control. In evaluating whether upregulation of DRM target genes occur with bias to specific cell types, our single cell analysis demonstrated that DRM target genes are broadly dysregulated throughout all identified cell types. Altogether, our results demonstrate that the AID system is a powerful and precise tool that enables the evaluation of the transcriptional dynamics that follow degradation of essential regulators like the DRM complex.

565C Tissue-specific incorporation of Clickable non-canonical amino acids in *C. elegans* via genetic code expansion

Rebekah J White, Qi Li, Sebastian Greiss University of Edinburgh

Genetic code expansion (GCE) allows incorporation of non-canonical (not naturally occurring) amino acids into proteins. GCE in *C. elegans* has previously been established by our research group, laying the foundation for non-canonical amino acid (ncAA) to be incorporated into worm proteins during translation. The technology utilises orthogonal aminoacyl-tRNA-synthetase (aaRS) / tRNA pairs that do not cross-react with any endogenous aaRSs or tRNAs.

We have used GCE to incorporate 'Clickable' ncAAs. A Click reaction is the highly selective coupling of two molecules, such our chosen ncAAs and a tetrazine molecule, with minimal byproduct. This means we can rapidly and temporally label proteins using tetrazine conjugated dyes, or use the Click reaction to isolate proteins from complex mixtures for further analysis such as mass spectrometry. sBy using orthogonal tRNA variants that decode sense codons we can stochastically label the proteome of specific cells and tissues where the orthogonal aaRS/tRNA pair is expressed. Unlike other methods for tissue specific proteome labelling in worms, such as BONCAT, our system does not depend on specific bacterial strains as a food source. It also allows the use of ncAAs capable of highly efficient and rapid copper-free Click-reactions and facilitates ncAA incorporation at any desired (or even multiple) sense codon.

We demonstrate this approach using three different tetrazine-Clickable ncAAs which we reliably and efficiently incorporate in live transgenic *C. elegans in vivo*. The ncAA containing proteins are then either fluorescently labelled or isolated for mass spectrometry. So far, we have achieved this via GCE with ubiquitous promoters, meaning the ncAAs are incorporated throughout the worm body. We are now working toward optimising the approach with tissue-specific promoters, which will result in rapid and selective tissue-specific and temporal *in vivo* protein labelling allowing tissue-specific proteomics.

566C Regulation of the age-dependent transcriptional activity of DAF-16 by the PTEN ortholog DAF-18

Matt Youngman, Elizabeta Berisha, Emma Burnham, Abigail Danner Biology, Villanova University

DAF-16/FOXO is a longevity determinant and suppressor of the extended lifespan of insulin receptor (*daf-2*) mutants. While normally maintained in an inactive state in young animals, environmental stress induces DAF-16 transcriptional activity. Beginning in the reproductive phase of life in *C. elegans*, however, DAF-16 activity increases in an age-dependent manner, even in unstressed worms. In post-reproductive animals, which lack the *pmk-1* branch of innate immunity due to immunosenescence, DAF-16 confers resistance to microbial pathogens. To understand how DAF-16 is regulated during aging, we tested the hypothesis that DAF-18, a dual specificity phosphatase that acts upstream in the insulin/IGF-1 (IIS) pathway, may be required for the dynamic activity of DAF-16 and, consequently, for DAF-16-mediated immunity in adults. While *daf-18*(*ok480*) mutants show a mild increase in pathogen susceptibility as larvae, this phenotype becomes more pronounced in post-reproductive adults, suggesting an increasing dependence on DAF-18 for immunity during aging. To confirm that DAF-18 regulates DAF-16 transcriptional activity during adulthood, we measured endogenous transcript levels of several DAF-16 targets in adult *C. elegans* by qRT-PCR and found that their age-dependent increase in expression requires DAF-18. Similar to its mammalian ortholog PTEN, the substrates of DAF-18 appear to include both phospholipids and phosphoproteins. Our functional analysis of *daf-18*(*yh1*) mutants, which lack lipid phosphatase activity but retain protein phosphatase activity, indicate that in its role in innate immunity in adult animals the primary enzymatic activity of DAF-18 is as a lipid phosphatase, consistent with its function in dephosphorylating PIP3 generated by AGE-1 in the IIS pathway. To confer immunity in adults, DAF-18 functions in the same tissues as DAF-16--the intestine and neurons. While undetectable in the intestine of post-reproductive animals, DAF-18::GFP and DAF-18::RFP fusion proteins are expressed robustly in a subset of head neurons. Specifically, we find that DAF-18 is expressed in AWC amphid neurons and at least two other neurons in the head. Work to identify these neurons and to further define the function of neuronal DAF-18 in innate immunity in adults is underway. Taken together, our data imply that DAF-18 may act in both a cell autonomous and non-autonomous manner to regulate age-dependent basal DAF-16 activity in adult *C. elegans*.

567C Examining how terminal nucleotide variations in miRNA duplexes influence strand selection throughout *Caenorhabditis elegans* development

Sarah Zhang, Jeff Medley, Sumire Kurosu, Anna Zinovyeva Division of Biology, Kansas State University

Regulation of gene expression enables various cellular and tissular functions that drive animal development. microRNAs (miRNAs) are small, non-coding RNAs that play key roles in regulating gene expression by silencing target genes. During miRNA biogenesis, miRNA precursors are processed into double-stranded duplexes. In a process referred to as strand selection, one miRNA strand is loaded into an Argonaute protein while the other is degraded. As the gene targets of miRNAs are determined by sequence complementarity to the Argonaute-loaded miRNA, each strand originating from the same duplex is expected to target a unique set of genes. While alternative miRNA strand selection may promote differential gene expression under physiological conditions, abnormal miRNA strand selection is often observed in human diseases, including many cancers. However, the regulatory mechanisms that might lead to altered strand selection are not fully understood. Previous studies suggest that the duplex ends of miRNAs play a role in determining strand choice. Argonaute favors miRNA strands containing a 5′ Uracil and appears to favor loading the less thermodynamically stable duplex end. In addition, preliminary data from our lab suggests that 3′ nucleotide identity also influences strand choice in *C. elegans.* As 3′ ends of miRNAs are often more dynamic than 5′ ends, regulation of 3′ nucleotide identity may serve as a mechanism to regulate strand selection. We hypothesize that changes in 3′ nucleotide identity should correlate to changes in miRNA strand selection. To address this, we quantified miRNA variants (isomiRs) throughout *C. elegans* development and analyzed whether variations in 3′ nucleotide identity were associated with altered strand ratios. We identified several changes in 3′ isomiR abundance that corresponded to changes in miRNA strand preference across developmental transitions. For example, we found that an isomiR of miR-60 that introduced an unfavorable 3′ nucleotide was three-fold more abundant in embryos than L1 larvae, which correlated to a nearly four-fold difference in the relative abundance of each miR-60 strand. Collectively, our findings indicate that changes in 3′ nucleotide identity may contribute to the regulation of strand preference throughout animal development and likely other contexts. These findings improve our understanding of alternative miRNA strand choice and may offer important insights into the dysregulation of strand selection in human diseases.

568C Cycloheximide resistant ribosomes reveal adaptive translation dynamics in *C. elegans*

Qiuxia Zhao, Blythe Bolton, Reiko Tachibana, Can Cenik, Elif Sarinay Cenik The University of Texas at Austin

Protein translation regulation is critical for cellular responses and development, yet how disruptions during the elongation stage shape these processes remains incompletely understood. Here, we identify and validate a single amino acid substitution (P55Q) in the ribosomal protein RPL-36.A of *Caenorhabditis elegans* that confers complete resistance to high concentrations of the elongation inhibitor cycloheximide (CHX). Heterozygous animals carrying both wild-type RPL-36.A and RPL-36.A(P55Q) exhibit normal development but intermediate CHX resistance, indicating a partial dominant effect. Leveraging RPL-36.A(P55Q) as a single-copy positive selection marker for CRISPR-based genome editing, we introduced targeted modifications into multiple ribosomal protein genes, confirming its broad utility for altering essential loci. In L4-stage heterozygotes, where CHX-sensitive and CHX-resistant ribosomes coexist, ribosome profiling revealed increased start-codon occupancy, suggesting early stalling of CHX sensitive ribosomes. Chronic CHX reduced ribosome collisions, evidenced by fewer disomes and unchanged codon distributions in monosomes. Surprisingly, prolonged elongation inhibition did not activate well characterized stress pathways–including ribosome quality control (RQC), the ribotoxic stress response (RSR), or the integrated stress response (ISR)–as indicated by absence of changes in RPS-10 ubiquitination, eIF2α phosphorylation, PMK-1 phosphorylation, or the transcriptional upregulation of ATF-4 target genes. Instead, RNA-normalized ribosome footprints revealed gene-specific changes in translation efficiency, with nucleolar and P granule components significantly decreased while oocyte development genes were increased. Consistent with these observations, we detected premature oogenesis in L4 animals, suggesting that partial translation elongation inhibition reshapes translation efficiency, to fine-tune developmental timing.

569C Exploring the Mechanisms of Transcription Regulation by Condensin DC and Cohesin in *C. elegans*

Alexandra Zhikharev, Haoyu Wang, Sevinç Ercan Biology, New York University

In eukaryotes, the transcriptional regulation of genes involves the 3D organization of DNA, which is governed in part by structural maintenance of chromosomes (SMC) complexes through making DNA loops. There are two types of SMC complexes associated with transcription regulation: cohesin, which is positively correlated with transcription, and condensin, which is associated with transcription repression. C. elegans has a third type of condensin, called condensin DC (dosage compensation), which is associated with repression of transcription specifically on the X-chromosome as part of dosage compensation. Preliminary data suggested that condensin DC reduces cohesin binding on the X-chromosome. This research will investigate how condensin DC represses transcription in C. elegans and how cohesin might be involved in this process. We will seek to distinguish between two modes of mechanism. The first is that condensin DC inhibits the activity of cohesin, and cohesin increases transcription levels of the X chromosome. Alternatively, condensin DC acts independent of cohesin to repress transcription, and cohesin is recruited to DNA by transcription increasing upon condensin DC depletion. To do a double knockdown of DPY-26, component of condensin DC, and SMC-3, component of cohesin, utilizing an auxin-induced degradation system, we made a strain homozygous for a GFP-degron insert on both genes, and confirmed using PCR and gel electrophoresis to test for the genotype. We incubated L3 larvae stage worms on auxin-containing plates for time intervals of 0h, 1h, and 2h, and performed fluorescence microscopy using the GFP signal to verify the knockdown. We extracted mRNA from each time interval and purified it to obtain mRNA-seq data to measure levels of transcription in each condition. We will use the data to distinguish between the two proposed mechanisms of action of condensin DC and cohesin, which will lead to further research to better understand their interaction. This interaction is important to understand since both chromosome compaction and gene expression are vital processes needed in order for organisms to develop properly.

570C Role of the RNA-binding protein UNK-1/Unkempt in dauer stage miRISC regulation

Julia Zhou[1], Himani Galagali[2], Amelia Alessi[1], Axel Schmitter[3], Isaac T. Smith[3], Jolene Diedrich[4], John R. Yates[4], Xantha Karp[5], John K. Kim[11]Johns Hopkins University, [2]Harvard University, [3]Central Michigan University, [4]The Scripps Research Institute, [5]Central Michigan University

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate post-transcriptional gene expression and play critical roles in development and disease. The Argonaute protein ALG-1 is the central component of the miRNA-induced silencing complex (miRISC), which recruits specific cofactors to modulate its activity in response to distinct cellular contexts, including development, stress, and aging. However, the mechanisms underlying miRISC remodeling and maintenance of developmental quiescence during environmental stress remain poorly understood. To investigate miRISC regulation during stress, we examined miRISC composition in dauer larvae, a stress-resistant developmental stage, using immunoprecipitation followed by mass spectrometry. This analysis identified two novel miRISC interactors: the conserved zinc finger RNA-binding protein UNK-1 (*Unkempt* in fly) and its binding partner, CRI-1 (*Headcase* in fly). UNK-1 expression is specifically and highly upregulated in dauer in a FOXO/DAF-16-dependent manner. RNA binding analyses reveal that UNK-1 associates with canonical *let-7* miRNA targets, suggesting substantial overlap between UNK-1 and ALG-1 miRISC target mRNAs. Consistently, *let-7* targets are upregulated in *unk-1(-)* mutant dauers, indicating that UNK-1 is required for efficient miRNA-mediated gene silencing. Supporting a role for UNK-1 as a negative regulator of *let-7* miRNA activity, *unk-1(-)* and *cri-1(-)* mutant dauers exhibit precocious exit from dauer, as evidenced by premature expression of the adult collagen marker COL-19::GFP. These findings suggest that UNK-1 and CRI-1 interact with miRISC to repress pro-developmental gene programs, including *let-7*-regulated pathways, facilitating stress adaptation in dauer. Future work aims to elucidate the molecular mechanisms by which UNK-1 and CRI-1 regulate miRISC function and developmental quiescence, including identifying UNK-1 mRNA targets, characterizing protein interactions between UNK-1, CRI-1, and ALG-1, and defining the regulatory mechanisms controlling UNK-1 and CRI-1 expression in response to stress.

571C TOFU-7 Facilitates Mitochondrial Coordination of piRNA Processing in *C. elegans*

Cole Pero, Craig C. Mello RNA Therapeutics Institute, University of Massachusetts Chan Medical School

Ensuring germline integrity across generations is essential for animal reproduction. One conserved mechanism involves PIWI-interacting RNAs (piRNAs), which regulate gene expression and protect genome stability. In *C. elegans*, many steps in piRNA biogenesis have been identified, including transcription and enzymatic processing of both transcript ends. However, how these steps are coordinated remains unclear. Here we investigate the role of TOFU-7, a KH-domain protein previously identified as a post-transcriptional regulator of piRNA biogenesis. We find that in tofu-7 mutants, the stability of the PIWI Argonaute, PRG-1 is greatly reduced and although they are still transcribed, very few piRNAs reach maturity. This suggests that TOFU-7 enhances the processing of piRNAs. Notably, unlike PRG-1 and other 3′ processing enzymes which are perinuclear, TOFU-7 localizes to mitochondria. This localization suggests a possible functional link with the 5′ processing PUCH complex, which is also mitochondrial. TOFU-7 lacks a transmembrane domain, instead carrying an N-terminal signal peptide. TOFU-7 retains its mitochondrial localization and function even when this peptide is removed, indicating a direct interaction with a mitochondrial protein, potentially a PUCH component, that can bypass the need for a localization signal. Despite its mitochondrial localization, TOFU-7's function does not depend on mitochondrial activity, as spg-7 RNAi-induced mitochondrial dysfunction does not impact TOFU-7 or PRG-1 stability. Our findings support a model in which mitochondrial localization of piRNA processing factors compartmentalizes processing steps for efficiency while maintaining proximity to P granules via the ubiquitous mitochondrial network. We speculate that TOFU-7 may play a role in facilitating the transfer of piRNA intermediates between mitochondrial and perinuclear processing steps. Further characterization of TOFU-7 will provide insights into the ordered regulation of piRNA biogenesis in *C. elegans*.

572C SUMOylation of Chromodomain Protein MRG-1 Regulates Cell-Fate Specification

Johan Girgenrath[1], Craig C. Mello[21]RTI, UMass Chan Medical School, [2]Umass Chan Medical School

MRG-1, a conserved chromodomain protein, regulates gene expression by binding methylated histone H3K36. It promotes germline gene expression while repressing transgenes and X-linked genes in *C. elegans*. MRG-1 interacts with both histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes, suggesting it recruits opposing chromatin-remodeling factors in a context-dependent manner. However, the mechanism underlying this regulation remains unclear.

We identify SUMOylation at lysine 301 (K301) as a key post-translational modification of MRG-1. *mrg-1(K301R)* mutants, which lack SUMOylation, exhibit increased sensitivity to NuRD complex depletion, leading to ectopic germline gene expression in somatic tissues and developmental arrest. Unlike *mrg-1* null mutants which suppress the larval arrest and soma-to-germline cell fate transformations caused by NuRD complex depletion, *mrg-1(K301R)* has the opposite effect enhancing the arrest phenotype while increasing the ectopic expression of germline genes in somatic tissues.

In contrast, during later development *mrg-1(K301R)* behaved similarly to *mrg-1* loss of function, suppressing the synthetic multivulval (*synMuv*) phenotype of *lin-15AB* mutants, *and* decreasing ectopic *lin-3* expression in hypodermal cells.

Immunoprecipitation (IP)/MudPIT analysis reveals that SUMOylation modulates MRG-1's interactions with chromatin regulators: *mrg-1(K301R)* preferentially associates with the Sin3S HDAC complex, while wild-type MRG-1 interacts more with the MYST HAT complex. These findings suggest that SUMOylation directs MRG-1's recruitment of chromatin-remodeling complexes, influencing cell-fate decisions in distinct developmental contexts.

These findings suggest that while MRG-1 is required for proper germline gene expression, its SUMOylation in adults and/or early embryos promotes NuRD complex functions necessary for the transition from germline to somatic fates. In early development, loss of SUMOylation enhances NuRD depletion phenotypes, leading to increased ectopic germline gene expression in somatic tissues and developmental arrest. However, during later development, both MRG-1 function and its SUMOylation are required in synMuv mutants to prevent ectopic vulval specification, likely through regulation of *lin-3* expression through recruitment of MYST complex components. Thus, SUMOylation serves as a critical regulatory mechanism that enables MRG-1 to differentially modulate chromatin-remodeling activities in distinct developmental contexts.

573A Bridging Inclusion and Scientific Inquiry: Teaching the Impact of Representation Through Genetics and Genomics Learning Modules

Olivia Davir[1], Quinn Tufiño[1], Madison Jones[1], Te-Wen Lo[1], Deborah M. Thurtle-Schmidt[21]Biology, Ithaca College, [2]Biology, Davidson College

For the field of genetics and genomics to equitably serve all members of society, both the scope of studies and its participants need to become more diverse and inclusive. Addressing this call begins in the undergraduate classroom. To confront equity in genetics education, we sought to design teaching modules highlighting the importance of inclusion practices in genetics research, providing students with the knowledge and tools to engage in studies that do as such. Here we outline a multi-part lesson to emphasize inclusion in the genetics classroom using both *C. elegans* and open-source genomics resources through centering representation in GWAS studies and using RNAi methods in worms to model rare genetic diseases. Specifically, this lesson highlights two modules, which can be used in concert or separately to best fit a variety of classroom environments. One module focuses on modeling rare human genetic disorders through RNAi in a *C. elegans* lab course, which has been adapted for both majors and non-majors genetics courses. A second module outlines a lesson over two class periods to outline Genome Wide Association Study (GWAS) methods and dive into data from the genome wide diversity index of representation within GWAS for students in an upper-level biology course. These lessons emphasize the importance of greater inclusion and incorporation of diverse populations, particularly historically excluded groups, addressing ethical implications in genetic and genomic research to expand the range of research topics in genetics but also our ability to accurately represent the human genetic landscape. By highlighting this inclusion in the course content we can increase a sense of belonging for all students creating a rightful presence in the classroom.

574A Bridging Research and Education with Model ORganisms (BREWMOR)

Paul D Goetsch[1], Te-Wen Lo[2], Dondra Bailey[3], Kelli Carroll[4], Sean Coleman[5], Peren Coskun[6], Rebecca Delventhal[7], Renee C. Geck[8], Julia Lee-Soety[9], Michael Law[10], Patricia Melloy[11], Kelsey Perry[12], April Rich[13], Adam Rudner[14], Josefa Steinhauer[15], Sumitra Tatapudy[161]Biological Sciences, Michigan Technological University, [2]Ithaca College, [3]Coppin State University, [4]Wofford College, [5]Wartburg College, [6]UMass Chan Medical School, [7]Lake Forest College, [8]Gonzaga University, [9]St. Joseph's University, [10]Stockton University, [11]SUNY New Paltz, [12]North Central Michigan College, [13]University of Pittsburgh, [14]University of Ottawa, [15]Yeshiva University, [16]University of Washington

BREWMOR (Bridging Research and Education With Model ORganisms) is an organization of educators and scientists united around our shared goal of building a network dedicated to increasing experiential learning for biology students primarily at the undergraduate level. BREWMOR disseminates best practices to engage and support students in rigorous model organism-based research excellence while creating and fostering strong, diverse and inclusive communities. We aim to connect with more researchers and educators within the GSA community to exchange resources, knowledge, and experience, and expand our community.

BREWMOR was founded following the 2020 TAGC meeting to help fulfill a need for educators around the globe in delivering hands-on, discovery-based undergraduate learning experiences during the height of the COVID-19 pandemic. The original work of BREWMOR was focused on high-impact teaching practices that engage students in an online learning environment.

BREWMOR's outreach has focused on the most pressing needs for educators. We host community-focused virtual workshops and seminars on topics at the intersection of research and education. These workshops, sponsored by GSA generally fall into two categories; a winter half-day workshop called a microBREW and a one- or two-day summer symposium and workshop called a bigBREW. Previous event topics have included: DEI Basics for Science Educators, CUREs, Grants for Education-Related Work and Research at PUIs, Integrating Primary Literature into the Classroom, and How to Work With AI in the Classroom.

We are actively working on ways to maintain engagement between workshops, including peer groups, and provide opportunities for community members to report back on their experiences in learning new pedagogy and implementing these new approaches in their classrooms. Come visit our poster to learn more about our community-building endeavors and future workshops.

575B Efficacy of a Course-Based Undergraduate Research Experience Focused on Biological Stress (StressCURE) in Biochemistry and Organic Chemistry Laboratory Courses

Jennifer L Newell-Caito[1], Sam Caito[2], Julie Hall[3], Mindy Reynolds[4], Jessica Hartman[5], Jade Schroeder[6], Joshua Gray-[71]Molecular & Biomedical Sciences, University of Maine, [2]Husson University, [3]Molecular Biology, Lincoln Memorial University, [4]Biology, Washington College, [5]Biochemistry and Molecular Biology, Medical University of South Carolina, [6]Marine Science, US Coast Guard Academy, [7]Chemical & Environmental Sciences, US Coast Guard Academy

Undergraduate exposure to research experiences in science is transformational, leading to benefits that include increased student retention in STEM, self-identification as a scientist, and progression towards graduate school. However, traditional undergraduate research experiences are difficult to employ on a large scale. Course-based Undergraduate Research Experiences (CUREs) are an effective way to expose undergraduates to research in the regular course of instruction throughout their educational program. StressCURE is an NSF-funded program supporting six faculty members to develop a modular approach to CURE design which focuses on the concept of biological stress in the *C. elegans* model system. Biological stress is defined as anything that disrupts the homeostasis of an organism, which may include exposure to toxicants, radiation, temperature extremes, diets, and genetic alterations. StressCURE was adopted in two courses in the Fall of 2024: a biochemistry laboratory at the US Coast Guard Academy and an organic chemistry laboratory at the University of Maine. Using the pathway model approach to program assessment, we linked key activities of the project with short-, middle-, and long-term outcomes and identified key assessment points needed to demonstrate programmatic success. Students were assessed using the Experimental Design Ability Test (EDAT) which is an open-ended response for students to design their own experiment to a prompt, before and after the course, assessed by the professor using a validated rubric. Students also took the laboratory course assessment survey (LCAS) to evaluate their perceptions of course which contained the StressCURE. Results of the EDAT showed an increased the ability to design experiments, identify variables, and explain how to apply statistics to experiments. As assessed by the LCAS survey, students enjoyed the collaboration involved in the StressCURE course and felt it provided the opportunity to actively participate in the scientific method. These data show the importance of utilizing CURE approaches like StressCURE for increasing critical student skills needed for their development as scientists. This material is based upon work supported by the National Science Foundation under Grant Number 2416714 as part of the Improving Undergraduate STEM Education (IUSE EDU). StressCURE course materials are freely available at stresscure.org.

576B Integrating worm research into undergraduate laboratory courses

Joseph Ross Biology, California State University, Fresno

Course-based undergraduate research experiences (CUREs) are increasingly common in universities because they democratize access for all students to authentic research practices. CUREs can also help faculty in many ways, including: recruiting students into mentored research, conducting large-scale research activities, and piloting high-risk high-reward project ideas to collect preliminary data. Here I share best practices (and pitfalls) from a decade of developing a CURE incorporating research in *Caenorhabditis* genetics for an undergraduate lab course in genetics and molecular biology. This course is taught at a large regional-serving public university that is a Hispanic-Serving Institution and an Asian American and Native American Pacific Islander-Serving Institution. Multiple sections of between 12 and 18 students are taught per semester, where small student teams are guided through the process of background reading, developing a hypothesis and experimental design, and then conducting an experiment and analyzing, interpreting and presenting their findings. I compare and contrast student opinions of the course and their attainment of learning outcomes, focusing primarily on differences arising from whether each group conducted their own project (self-directed projects) or decided to work with the other groups in class to conduct one project distributed among the class sections. A key result is that the vast majority of students report wanting to have this sort of laboratory course experience earlier in their undergraduate career. This course design also lends itself to conducting research replication to validate published studies, which provides a valuable service.

577B The *C. elegans*-based "Pipeline CURE" provides apprentice-style research benefits for diverse cohorts of Biology students at a primarily undergraduate institution and a partner Title 1 high school

Karen L. Schmeichel[1], Onur Birol[2], Lisa J. Hayes[3], Brandon S. Carpenter[4], Teresa W. Lee[5], David J. Katz[61]Oglethorpe University, [2]College of Sciences, Georgia Institute of Technology, [3]Behavioral Sciences Division, Oglethorpe University, [4]Molecular and Cellular Biology, Kennesaw State University, [5]Biological Sciences, U. Mass - Lowell, [6]Cell Biology, Emory University

High impact STEM instruction in higher education increasingly relies on the learning opportunities afforded by genuine research experiences. Course-based Undergraduate Research Experiences (CUREs) involve larger numbers of undergraduate students than traditional apprentice-style research programs and thus show promise in improving access to, and inclusivity in, STEM career training. Single semester CUREs have limited capacity to support the deep learning and gradual workplace acculturation afforded by long-term, iterative research. For the last 15 years a scaffolded, multi-course "Pipeline CURE" has been supported by a longstanding partnership between two universities in the Atlanta area: Oglethorpe University (a small majority-minority Primarily Undergraduate Institution) and Emory University (a private Atlanta-area R1 research hub). Students trained in the Pipeline CURE perform authentic research, over a four-course arc, on epigenetic reprogramming in C. elegans. Data collected from both attitudinal surveys and concept inventories at every stage of the Pipeline CURE show increased student efficacy and sense of belonging in STEM that emerges in a distinctly non-linear fashion. The Pipeline CURE has been effective in guiding its participants directly into STEM research positions and M.S and Ph.D. granting programs. Thus, the Pipeline CURE recapitulates a significant number of benefits associated with traditional apprentice-style research experiences. Importantly, Pipeline CURE students, most of whom are juggling jobs, school and significant family responsibilities, gain these benefits entirely within a normal undergraduate curriculum. To provide "on-ramps" to the Pipeline CURE, the program has been expanded to include a cohort of early career high school students from an Atlanta area Title I public high school. Oglethorpe Pipeline CURE undergraduates come from identity groups that mirror those of high school participants, and thus they serve as approachable and effective near-peer mentors for younger trainees. These two groups of students work collectively on research projects for both high school science fair competitions and for undergraduate Honors theses. Our findings have significant implications for improved access to STEM training both before, and at, the college level.

578A Exploring Genes Required to Make an Exopher from Proteostressed Neurons

Mark Abbott, Ilija Melentijevic, Ryan Guasp, Monica Driscoll Genetics, Rutgers

Elucidating neuroprotective mechanisms that limit proteotoxicity is an important goal in the battle against neurodegenerative disease. We discovered, and have begun to characterize, a previously unknown capacity of *C. elegans* adult neurons to extrude large (~5μM) vesicles that include deleterious cell contents. We call these extruded vesicles exophers. Inhibiting chaperone expression, autophagy, or the proteasome, as well as over-expressing aggregating proteins like human AD Ab1-42, expanded polyglutamine Q128 protein, or high concentration mCherry increases exopher production from the affected neurons. Extruded exopher contents can be found in both neighboring and remote cells. We hypothesize that "throwing out the trash" (exophergenesis) is a conserved mechanism that constitutes a fundamental, but formerly unrecognized, branch of neuronal proteostasis. The analogous process in mammals could promote transfer of misfolded protein to neighboring cells contributing to human pathology in neurodegenerative disease.

The genetic mechanisms involved in exopher generation are largely unknown. Although the mechanistic dissection of large vesicle aggregate extrusion in a genetic model like *C. elegans* holds tremendous potential for generating insight into this underexplored biology, there are some challenges. As first characterized, we found exophers are produced relatively infrequently, and without any available markers specific for exopher production, non-biased genetic screens are exceptionally difficult. Using a highly automated whole genome screening method developed by our lab, we were able to perform a whole genome screen for touch neuron exopher modulation using a *sem-2* knockout strain. The *sem-2* strain has high egg retention, which has a large stimulatory effect on exopher production rates, making a screen in a neuronal RNAi-sensitized strain (especially for suppressors) possible. Our analysis of screen results elucidates how exophergenesis is integrated with proteasome, autophagy and germline biology to modulate exopher production.

579A High-Resolution Spatial Transcriptomics and Automated Neuron Annotation in *C. elegans* via seqFISH

Jose David Aguirre Aguilera, Xuan Wan, Carsten Tischbirek, Alessandro Groaz California Institute of Technology

The C. elegans nervous system, comprising 302 neurons and 56 glial cells in the hermaphrodite, provides a well-defined model for studying neural circuits and their development. We have developed a protocol to use sequential in situ hybridization (seqFISH) to perform high-resolution spatial transcriptomics in C. elegans. We use set of well-characterized marker genes to accurately annotate individual neurons in both sexes, enabling precise identification of the cells expressing the transcripts we profile. We have screened 120 candidate genes to add to these markers and want to identify a set of 20 genes that will let us robustly annotate >90% of neurons.

Because manual neuron annotation is labor-intensive and requires detailed anatomical expertise, we are developing a computational pipeline for automated neuron identification. By training a machine-learning model on marker gene expression and anatomical features, we will automate neuron annotation and transcript quantification. This strategy will enhance accuracy, reproducibility, scalability, and efficiency in single-cell spatial transcriptomics, facilitating broader applications in neural circuit research in C. elegans and other nematodes. We are applying seq-FISH to identify additional genes expressed in male-specific neurons, to analyze the phenotypes of undead cells, and to examine plasticity.

580A Human-associated odorants drive host invasion in the human-infective, skin-penetrating nematode *Strongyloides stercoralis*

Damia Akimori[1,2], Aaliyah Tinoco[1], Michelle L Castelletto[1], Elissa A Hallem[1,3]1Microbiology, Immunology, and Molecular Genetics, UCLA, [2]Molecular Biology Interdepartmental Ph.D. Program, UCLA, [3]Molecular Biology Institute, UCLA

Over 610 million individuals globally are infected with the skin-penetrating intestinal parasitic nematode *Strongyloides stercoralis*. *S. stercoralis* infect hosts exclusively as developmentally arrested infective third-stage larvae (iL3s). The iL3s navigate through the soil searching for hosts in a poorly understood process that involves attraction to host-emitted olfactory cues. Upon locating a host, iL3s invade by burrowing through the skin in another process that may involve attraction to host-associated olfactory cues. We have identified a subset of human-associated odorants that stimulate both host-seeking and skin-penetration behaviors of *S. stercoralis* iL3s as well as odorants that independently stimulate either host seeking or invasion. Furthermore, the responses to these odorants are species-specific – they do not stimulate skin penetration in the closely related rat-parasitic nematode *Strongyloides ratti.* We are now investigating the neural and molecular mechanisms that underlie these responses. Large families of G protein-coupled receptors encode odorant receptors (ORs) in nematodes and have been thoroughly identified in the model nematode *Caenorhabditis elegans*. We verified and manually curated putative OR genes in *S. stercoralis*. Through analysis of differential gene expression between iL3s and free-living life stages of *S. stercoralis*, we identified several OR genes that are highly expressed and upregulated in iL3s. Fluorescent reporter constructs using the promoters for these OR genes revealed expression in specific subsets of head sensory neurons, enabling us to genetically target putative olfactory neurons in *S. stercoralis* for the first time. We are now testing the requirement for these neurons during skin penetration using chemogenetic silencing and examining their activity using calcium imaging. Together, these findings lay critical foundations for untangling mechanisms of parasitic nematode invasion and consequently may identify novel therapeutic targets for their inhibition.

581A Characterizing multiple mechanosensory behaviors through development

Ilana Albert[1], Sina Rasouli[1], Alyssa Goldstein[2], Jinmahn Kim[1], Mahdi Torkashvand[1], Vivek Venkatachalam[1]1Physics, Northeastern University, [2]Biochemistry, Northeastern University

Mechanosensation is a critical process in allowing animals to avoid danger. Based on the collective work across many labs, we have a good sense of mechanosensory evoked behaviors in worms including mechanosensory response rates, sensitivity, timing of escape behaviors, and habituation of mechanical responses over time. In ethological studies looking at escape from predatory fungi, it has been found that efficient escape responses are age dependent [Maguire et al. 2011]. Additionally, the connectome suggests that cells involved in the escape response change their wiring through development [Witvliet et al. 2021]. This has motivated us to more carefully characterize mechanically induced escape behaviors through development.

We probed this question using three mechanosensory assays at all developmental stages. (1) We nonspecifically tapped whole plates of animals stimulating both anterior and posterior touch neurons. (2) We optogenetically activated soft touch neurons - ALM, PLM, AVM, PVM - using Chrimson. (3) We touched the heads and tails of individual animals and imaged the calcium activity in corresponding mechanosensory neurons. Using these three methods we were able to characterize – across developmental stages – how different mechanical stimuli are sensed, and how head and tail signals may be integrated to drive mechanically induced behavior.

582A Aberrant neuronal hyperactivation causes age-dependent thermotaxis decline

Binta Maria Aleogho[1], Mizuho Mohri[2], Moon Sun Jang[2], Sachio Tsukada[2,3], Yana Al-Hebri[2,4], Hironori J. Matsuyama[2], Yuki Tsukada[2,5], Ikue Mori[2,6], Kentaro Noma[21]Biological Science, Nagoya University, [2]Nagoya University, [3]MEG MILK SNOW BRAND, [4]New York University, [5]Keio University, [6]Chinese Institute for Brain Research

Aging induces a wide range of systemic alterations in animals, including deficits in sensory perception, learning, memory, cognition, and behavioral functions—symptoms generally attributed to neuron loss, synaptic dysfunction, and diminished neuronal activity. Concurrently, increased neuronal activity during aging is reported in humans and other organisms. Whether such neuronal hyperactivity contributes to behavioral impairment or acts as a compensatory mechanism for circuit dysfunction remains unclear. *Caenorhabditis elegans* exhibits an age-dependent decline in thermotaxis—an associative learning behavior involving the animal's temperature preference along a thermal gradient, conditioned by food availability during its cultivation. By neuronal ablations and calcium imaging, we demonstrate that the primary thermosensory circuit, comprising AFD sensory neurons and AIY interneurons, remains relatively intact during aging. On the other hand, the ablation of either AWC sensory neurons or AIA interneurons preserved thermotaxis ability in aged animals. In addition, we showed that AWC ablation suppressed aberrant reversals and improved curve biases in aged animals. Calcium imaging further revealed that both AWC and AIA neurons exhibit age-dependent, spontaneous, and stochastic hyperactivity, which is enhanced through reciprocal signaling between both neurons, differentially mediated by *unc-13* and *unc-31*. The age-dependent thermotaxis decline and neuronal hyperactivity were ameliorated by changing diets from *Escherichia coli* to *Lactobacillus reuteri*. Furthermore, we show that the ameliorative effect of *Lactobacillus reuteri* requires DAF-16 functioning in AWC neurons. Our findings establish a causal relationship between neuronal hyperactivity and behavioral decline during aging, highlighting the potential of dietary interventions to attenuate age-associated behavioral decline by targeting neuronal hyperactivity. We propose that aberrantly enhanced, rather than diminished, neuronal activity can impair the behavior of aged animals.

583A Investigating the molecular mechanisms through which age-associated neuronal lysosomal dysfunction promotes synaptic dysfunction.

Manuel Alvarez, Ruiling Zhong, Nikita Shiliaev University of Wisconsin-Madison

Synaptic dysfunction is a key driver of cognitive decline in aging, yet the mechanisms underlying age-associated synaptic decline remain unclear. Another hallmark of aging is lysosomal dysfunction, which is also strongly linked to neurodegenerative disease. We hypothesized that inadequate lysosomal function contributes to synaptic dysfunction in normal aging, in the absence of neurodegenerative diseases. Using fluorescent reporters to visualize the abundance and acidity of lysosomal compartments, we found that neuronal lysosomal function declines with age in wild-type *C. elegans*. We next aimed to identify how neuronal lysosomal degradative capacity (LDC) is regulated. Transcription Factor EB (TFEB)/HLH-30 is a known regulator of lysosome biogenesis, particularly under conditions of starvation and stress. We found that HLH-30 is dispensable for neuron development but functions in healthy, well-fed neurons *in vivo* to expand neuronal LDC during early adulthood, which delays the onset of lysosome dysfunction. We next asked whether HLH-30is required for proper neurotransmission. Pharmacological interrogation of synaptic activity combined with genetic analysis indicates that HLH-30 functions in both the pre- and post-synaptic cells to maintain proper neurotransmission in adulthood. We hypothesized that inadequate LDC in the *hlh-30* mutant leads to accelerated decline of neurotransmission through disrupted synaptic protein turnover. To begin to test this model, we first tested whether HLH-30 promotes synaptic protein turnover by developing the ARGO (Analysis of Red-Green Offset) method. ARGO co-translationally labels the protein of interest with both RFP and GFP in a tandem tag in specified cells via FLP/FRT. The steady-state GFP/RFP ratio indicates degradation mechanisms: GFP fluorescence is quenched in acidic lysosomal compartments, making endosomes appear RFP-enriched. A "pulse" removes *gfp* from the ARGO gene cassette, tagging newly synthesized proteins with only RFP. Fluorescence microscopy is then used to track GFP/RFP ratios across the cell over time, quantifying the proportion of "old" versus "total" protein spatially and temporally. We found that loss of HLH-30 increases synaptic vesicle protein half-life at the presynapse. As degradation and replacement of synaptic vesicle proteins are vital for neurotransmission and neuronal function, we speculate that this slowed turnover contributes to age-associated synaptic dysfunction. Moving forward, we will use ARGO to investigate how LDC regulates synaptic vesicle protein turnover, bridging the gap between LDC and age-associated synaptic decline.

584A Optogenetic Investigation of the Cause of Response Decrement to a Mechanical Stimulus Delivered at Short and Long Interstimulus Intervals

Vania Amani[1], Nikolas Kokan[1], Catharine Rankin[1,2]1Djavad Mowafaghian Centre for Brain Health, University of British Columbia, [2]Dept. of Psychology, University of British Columbia

Habituation is a simple type of learning characterized by a decrease in responsiveness to a repeated stimulus not caused by fatigue or sensory adaption. A conserved characteristic of habituation is how the time between stimuli presentations, known as the interstimulus interval (ISI), affects the rate, depth and memory of an animal's habituation. Organisms habituate faster and to a greater extent at shorter ISIs, while the memory of habituation is better at longer ISIs. However, sensory adaptation processes could influence response decrements at short ISIs. Sensory adaptation is a physiological phenomenon where the signal transduction process becomes increasingly insensitive to a repeated stimulus. In contrast, habituation is a learning process that occurs downstream of sensory transduction. Thus, at shorter ISIs, an organism may become less sensitive to a stimulus repeated as a result of adaptation of its sensory receptors, whereas at longer ISIs, the receptors can recover between stimuli. In *Caenorhabditis elegans*, habituation mechanisms have been studied at different ISIs by monitoring the animals' reversal responses with respect to mechanical taps to their Petri dish side wall. Mechanical taps involve stimulation of the worms' mechanosensory neurons; therefore, adaptation of mechanoreceptors could be causing the decreased responsiveness observed in short ISI experiments.

This work addresses whether the decrements in *C. elegans* reversal response result from sensory adaptation or are further downstream, indicating habituation. To bypass sensory adaptation, we used blue light to artificially stimulate Channelrhodopsin2, a light-gated ion channel that we have expressed in the worms' mechanosensory neurons, at short (10s) and long (60s) ISIs. Using our Multi-Worm Tracker, we compiled behavioral data from adult worms on Petri dishes, simultaneously tracking 50-100 animals. Reversal responses to blue light were compared with wild-type responses to mechanical tapping. We found that mechanical (tap) and nonmechanical (blue light) stimulation elicited similar reversal response decrements at both short and long ISIs. Our results demonstrate that the characteristic effect of ISI on habituation can be achieved in the absence of sensory adaptation. The findings from these studies will advance our understanding of how repeated mechanical stimuli impact *C. elegans* nervous systems and validate this procedure for future studies investigating habituation.

585A Investigating genetic modifiers in a novel *C. elegans C9orf72* ALS/FTD model

Selamawit S Asfaw, Mika Gallati, Alexander Lin-Moore, Jeremy Lins, Ashley Guo, Anne C. HartNeuroscience, Brown University

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of motor neurons in the brain and spinal cord. While most cases occur sporadically, several genes have been associated with familial ALS. Among these, a G4C2 hexanucleotide repeat expansion in *C9orf72* is the most common genetic cause of ALS and Frontotemporal Dementia (FTD), collectively known as ALS/FTD. Healthy individuals typically have fewer than 11 repeats, whereas ALS/FTD patients have anywhere from 30 to several thousand repeats, with symptom onset occurring in older adults. Repeat expansions in the *C9orf72* gene undergo repeat-associated non-AUG (RAN) translation, producing toxic dipeptide repeat proteins (DPRs) that may contribute to neurodegeneration through protein aggregation and impaired nucleocytoplasmic transport. However, the exact mechanisms underlying *C9orf72*-associated neurodegeneration remain unclear. To better understand these mechanisms, we developed a *C. elegans* G4C2 model that expresses 30 hexanucleotide repeats. These animals exhibit neuronal process degeneration in phasmid neurons, offering a clear and measurable indicator of neurodegeneration. Using this model, we have begun to test candidate genetic modifiers, drawing from both published studies and ongoing, unpublished research in our group. Additionally, we are testing different alleles of two suppressor genes from a screen performed using a *sod-1* ALS model to determine whether their protective effects extend to other ALS/FTD models, including the G4C2 model. By uncovering genetic pathways that influence *C9orf72*-linked toxicity, this work will improve our understanding of the mechanisms underlying ALS and potentially inform the development of future treatments.

586A Does sleep affect age-dependent locomotory decline?

Christopher D Ashih, Casey Tsimbal, Javier Apfeld Biology, Northeastern University

Much like humans and other model organisms, *Caenorhabditis elegans* exhibits decreased mobility with age. Some long-lived mutants display lethargy early in life. To investigate the mechanisms underlying this reduced mobility, we are crossing sleep-disrupted mutants with lifespan-extending mutations. Specifically, we are investigating the sleep-active neuron RIS and its interactions with partial-loss-of-function mutations in the insulin/IGF-1 receptor *daf-2* and null mutations in the phosphatidylinositol-3 kinase *age-1*, which extend lifespan by two- and eightfold, respectively. RIS is active during a period of *C. elegans* larval development termed lethargus, which precedes molting and is analogous to sleep. To turn RIS on or off, we are using strains engineered to inhibit or overactivate RIS through the expression of mutant forms of specific potassium and sodium channels1. To monitor locomotion in adult worms we developed a novel scanning and tracking setup, and modified existing methods previously used to track worms during development. We found distinct behavioral phenotypes in RIS-on and RIS-off adults, characterized by speed distribution and track pattern. These phenotypes appear to be maintained in *daf-2* mutants. We will present our ongoing characterization of these phenotypes at the worm meeting.

1Bringmann lab

587A Sex differences in context-dependent behavioral flexibility in *C. elegans*

Chance Bainbridge, Douglas S Portman Biomedical Genetics, University of Rochester Medical Center

Animals must constantly integrate external sensory cues with internal-state information to optimally select adaptive behavioral strategies. Often deployment of these behavioral strategies is flexibly modulated and tuned to increase fitness. Because nutritional requirements and reproductive strategies often differ by sex, selection of adaptive behavioral strategies during nutrient stress are often sexually dimorphic. Previous work from our lab and others indicates that *C. elegans* exhibit sexually dimorphic behavioral and neuronal responses to some nutrient-derived cues. However, the mechanisms by which biological sex impinges on underlying neuronal circuit logic to orchestrate appropriate nutrient-dependent behavioral strategies remains poorly understood.

Here, we investigate sex differences in behavioral responses to nutrient stress by profiling changes in locomotion in fed and fasted hermaphrodites and males. In hermaphrodites, food encounter causes a reduction in speed ("basal slowing") that is exaggerated in food-deprived animals ("enhanced slowing"). Whether biological sex impinges on the circuit logic underlying this integration between external and internal cues is unknown. To investigate this, we compared shared and sex-specific behavioral responses under food encounter context using a combined behavioral, optogenetic, and mutant analysis approach. Our results indicate that males exhibit basal slowing but lack nutrient-dependent enhanced slowing. Optogenetic stimulation of the serotonergic food-sensing NSM neuron, which reduces speed in hermaphrodites, had a reduced effect on speed with a faster recovery time in males. These data indicate that sex-specific regulation of neuronal function may be occurring downstream of NSM. In the longer term, our approach provides a unique opportunity to explore mechanisms by which context-dependent modulation of circuit logic can bring about flexible and adaptive behavioral strategies.

588A The Hox transcription factor *mab-5* is necessary and sufficient for transcriptional differences between left/right Q-derived neuron pairs in *Caenorhabditis elegans*

Talmage Barney[1], Alexis Rolfson[2], Samuel Bennett[2], Emma Davis[1], Mason Lyman[1], Seth R Taylor[1,2]1Neuroscience Center, Brigham Young University, [2]Cell Biology and Physiology, Brigham Young University

Neurons share a common core genetic program but also display striking diversity in morphology and function. We have used the post-embryonic neurons derived from the left and right Q cells of *Caenorhabditis elegans* as a model to understand the generation of transcriptional diversity in the nervous system. We describe previously undocumented transcriptional asymmetries between the left and right SDQ neurons. Single-cell RNA sequencing of adult animals revealed hundreds of differentially expressed genes between SDQR and SDQL, including several genes encoding neuropeptides and neuropeptide receptors. Using confocal imaging of endogenously tagged reporter alleles, we have validated differential expression of two neuropeptides: *nlp-64* in SDQR and the insulin-like neuropeptide *ins-17* in SDQL. These patterns of differential expression are established in the first larval stage, shortly after the SDQ neurons are generated, and persist into adulthood. We show that the Hox transcription factor *mab-5,* normally expressed in SDQL but not SDQR, is both necessary and sufficient for asymmetric expression of *nlp-64*. *nlp-64* is expressed in both SDQR and SDQL in *mab-5* loss of function mutant animals. Furthermore, *mab-5* is continuously required to maintain differential expression, since degradation of *mab-5* after initial development results in de-repression of *nlp-64* in SDQL. Ectopic expression of *mab*-5 in SDQR results in a loss of *nlp-64* expression. In addition, we show that *mab-5* is required for the differential gene expression of *gcy-35* between AVM and PVM, the sisters of the SDQ neurons. Loss of *mab-5* results in *gcy-35* expression in PVM. Together, these data show a continuous requirement for Hox gene expression in maintaining cell identity.

589A DEET inhibits skin penetration in a skin-invading, human-parasitic nematode

Gloria Bartolo[1,2], Michelle L Castelletto[1], Elissa A Hallem[1,3]1Department of Microbiology, Immunology and Microbial Genetics, University of California, Los Angeles, [2]Molecular Biology Interdepartmental PhD Program, University of California, Los Angeles, [3]Molecular Biology Institute, University of California, Los Angeles

Strongyloides stercoralis is a skin-penetrating, human-parasitic nematode that infects over 600 million people globally and can be fatal for immunocompromised individuals. While current medications help clear existing infections, they do not prevent infection and reinfection rates remain high in endemic areas. Although topical repellents are widely used to reduce the spread of insect-borne diseases, the possibility of using topical repellents to prevent nematode infections has not been investigated. We tested whether N, N-diethyl-meta-toluamide (DEET), a widely used insect repellent, affects the ability of *S. stercoralis* infective larvae to invade hosts by skin penetration. We performed *ex vivo* skin penetration assays using excised, epilated rat skin. Individual infective larvae were placed on rat skin evenly coated with either 30% or 50% DEET. Skin-penetration behaviors were then video-recorded and analyzed post hoc. We found that applying either 30% or 50% DEET to the skin surface greatly reduced skin penetration – whereas ~85% of the infective larvae penetrated into control skin, only ~30% of the larvae penetrated into DEET-coated skin. Moreover, the average time until the completion of penetration was delayed ~2-fold. Infective larvae exposed to DEET also exhibited novel behaviors on the skin surface, such as idling and looping. We then extended our behavioral analysis to human skin samples and found that DEET similarly reduced penetration on human skin. Lastly, to determine the effect of DEET on skin penetration in the presence of other external cues such as body heat, we performed *in vivo* skin penetration assays on anesthetized rats. Compared to a control condition where 85% of infective larvae penetrated the skin, not a single worm penetrated skin coated with 30% DEET. We are currently investigating the molecular mechanisms by which DEET blocks skin penetration. Our results identify DEET as a strong candidate for parasitic nematode control and prevention.

590A Visualizing neuropeptide signaling using GPCR activation reporters

Jan Watteyne[1], Pearl Thijssen[2], Lan Geng[3], Xinyu Huang[4], Anuj Sharma[2], Majdulin Nabil Istiban[1], Han Wang[4], Yulong Li[3], Andrew Leifer[2], Isabel Beets[11]KU Leuven, [2]Princeton University, [3]Peking University, [4]University of Wisconsin-Madison

Neuropeptides are signaling molecules central to the biology of all animals that mainly exert their effects through G protein-coupled receptors (GPCRs). Using reverse pharmacology and network analysis, we recently characterized the network of neuropeptide-GPCR interactions in the *C. elegans* nervous system [1, 2]. The wireless neuropeptide network has a different topology and is potentially more connected than the synaptic network. However, which neuropeptidergic connections are accessible and when has yet to be determined, since little is known about the spatial scope and temporal dynamics of neuropeptide signaling.

To address this, we are implementing genetically-encoded reporters for GPCR activation in *C. elegans.* We have developed a light-gated transcriptional reporter, "PepSee", that couples ligand-induced GPCR activation to the production of a stable, fluorescent signal by release of a GPCR-tethered transcription factor. We use PepSee to identify receptor-expressing target cells of peptidergic neurons upon spontaneous or optogenetically-induced release of neuropeptides. For example, tagging the neuropeptide receptor AEX-2, which controls the rhythmic defecation motor program upon binding of the gut-derived peptide NLP-40, with the PepSee cassette shows reporter activation in neurons where AEX-2 is known to function in defecation. Other neuronal and non-neuronal cells also show ligand-dependent reporter activity, but a biological role for NLP-40/AEX-2 signaling in these cells has yet to be discovered.

Besides transcription-based GPCR activation reporters, we are also employing real-time GRAB (*G* protein-coupled *R*eceptor-*A*ctivation-*B*ased) sensors to acquire high-resolution, time-resolved mapping of peptide diffusion dynamics in *C. elegans*. Expression of GRAB sensors in specific circuits or throughout the nervous system allows for real-time measurements of receptor binding. We then excite peptidergic neurons to release peptides, using optogenetics or sensory stimulation, while conducting high-speed imaging to track the diffusion of peptides through the worm. Altogether, these tools can act as a scaffold to investigate how the neuropeptide connectome is spatio-temporally organized and how flexible behaviors emerge from neuromodulatory networks.

[1] Ripoll-Sánchez *et al.*, *Neuron*. 2023 [2] Beets *et al., Cell Rep.* 2023

591A MIG-6/papilin mediates long-term maintenance of neuronal architecture through the regulation of extracellular matrix organization and TGF-β signaling

Malika Nadour[1,2], Robert Ivan Valette Reveno Leatis[2,3], Marie Biard[2,3], Noémie Frébault[2,3], Lise Rivollet[2,3], Philippe St-Louis[2,3], Cassandra R. Blanchette[4], Andrea Thackeray[4], Paola Perrat[4], Carlo Bevilacqua[5], Robert Prevedel[5,6], Georgia Rapti[5,7], Maria Doitsidou[8], Claire Benard[1,2,91]Universite du Quebec a Montreal, [2]CERMO-FC Research Center, [3]Université du Québec à Montréal, [4]University of Massachusetts Chan Medical School, [5]European Molecular Biology Laboratory, [6]Heidelberg University, [7]FENS-KAVLI Network of Excellence, [8]University of Edinburgh, Centre for Discovery Brain Sciences, [9]UMass Chan Medical School

Neuronal architecture established embryonically must persist lifelong to ensure normal brain function. However, little is understood about the mechanisms behind the long-term maintenance of neuronal organization. In cell-adhesion molecule mutants *sax-7/L1CAM*, distinct neuronal structures that develop normally later become disorganized. To uncover maintenance mechanisms, we performed a suppressor screen in *sax-7* mutants, which exhibit progressive disorganization with age. We identified the conserved extracellular matrix protein MIG-6/papilin as a key regulator of neuronal maintenance. MIG-6/Papilin harbors a papilin cassette, present also in metalloproteinase ADAMTS, which we show is required for neuronal maintenance. *mig-6* short isoform functions post-developmentally from muscles to non-autonomously function in neuronal maintenance, in a *mig-17*/ADAMTS-dependent manner. Interestingly, loss of *mig-6* leads to the accumulation of extracellular collagen IV fibrotic structures. Collagen IV levels and crosslinking are required for the suppression of *sax-7* neuronal defects by *mig-6* mutation, as the post-developmental depletion of collagen IV, or of its crosslinking enzyme peroxidasin, reinstates neuronal maintenance defects in *sax-7; mig-6* mutants. This remodeling impacts tissue biomechanics and ensures neuronal stability, even under increased mechanical stress. To determine how this *mig-6*-dependent fibrotic phenotype might be modulated, we investigated the TGF-β pathway, which is well known to regulate fibrosis in mammals. We find that TGF-β impacts collagen IV remodeling in the worm and participates in neuronal maintenance. Indeed, in *mig-6* mutants, loss of TGF-β signaling leads to increased collagen IV fibrotic structures accumulation and enhances the suppression of *sax-7* neuronal defects. Consistent with this, TGF-β components overexpression counteracts the suppression of *sax-7* neuronal defects by *mig-6*. Finally, other extracellular matrix components, such as laminin and fibulin, are also important for the *mig-6*-mediated suppression of *sax-7* neuronal defects and act in a TGF-β pathway-dependent manner. Altogether, our findings highlight an extracellular mechanism by which MIG-6 supports the integrity of neuronal architecture on the long term. This work provides critical insights into the molecular basis of sustaining neuronal architecture and offers a foundation for understanding age-related and neurodegenerative disorders.

592A Investigating the role of the long chain polyunsaturated fatty acid eicosapentaenoic acid (EPA) in modulating acute functional tolerance to ethanol

Jill Bettinger, Tyler Crossen, Laura Mathies, Yang Yue, Lauren Ashley Cowart, Andrew Davies Virginia Commonwealth University

We study the molecular machinery underlying the acute physiological response to ethanol (EtOH) because the level of response to EtOH in humans is strongly associated with the liability to develop alcohol use disorder. We use locomotion to test both initial sensitivity and the development of acute functional tolerance (AFT) to EtOH. We have found that the levels of endogenously generated or exogenously supplied (through diet) omega-3 long chain polyunsaturated fatty acid Eicosapentaenoic Acid (EPA) modulates the development of AFT; *fat-1* mutants do not generate EPA and cannot develop AFT, and this is rescued by dietary supplementation of EPA but not the EPA precursor Arachidonic Acid, indicating that EPA has a specific role in AFT (PMID: 25162400). This effect of EPA is conserved in mammals; feeding mice EPA-rich diets changed acute EtOH responses and consumption behaviors in a genotype-specific manner (PMID: 29786878), and genetic variation in genes involved in regulation of EPA levels are associated with AUD and AUD-related phenotypes in humans (PMID: 35904282). We are working to understand the mechanisms underlying this effect of EPA. Our working model is that EPA is acting in AFT through actions on membrane biophysics to alter membrane-bound protein activity. To test this model, we first conducted a careful time course of the effects of EPA on AFT. We begin to see an effect of EPA dietary supplementation by 10 hours of exposure, and full rescue of the EPA-deficient *fat-1* defect in AFT requires 14 hours of feeding. We tested the possibility that it takes this long for the animals to eat *enough* EPA to rescue AFT. We fed EPA to *fat-1* mutants for only 4 hours, removed the EPA, and then tested them at the 14-hour timepoint. This 4 hour bolus of EPA was sufficient to provide rescue, strongly suggesting that EPA must be metabolized, modified, or incorporated over this time to function in AFT. We have taken a combination of unbiased genetics and lipidomics approaches to identify which genetic pathways and EPA-containing molecules are important in this process. We performed a comprehensive transcriptional analysis using RNA-sequencing of wild-type and *fat-1* mutant animals with and without dietary supplementation of EPA over the time course of rescue. We asked for genes with these characteristics: First, the expression of a candidate EPA-responsive gene that is involved in AFT should differ between untreated N2 and *fat-1* mutants, and second, it should be responsive to EPA feeding in *fat-1* mutants. Fifty seven genes meet these criteria; we are testing these for effects on AFT. Second, we used d5-labeled EPA to rescue the *fat-1* AFT defect and then used lipidomics to identify d5-EPA-containing lipids at the time of rescue; among these should be the mediators of the effect on AFT. Together, these studies will contribute to our understanding of how omega-3 polyunsaturated fatty acids modify the acute behavioral response to EtOH.

593A Uncovering the neuronal basis of individuality in decision-making across development

Smriti Bhardwaj, Shay Stern Faculty of Biology, Technion, Israel Institute of Technology

Despite being genetically identical and exposed to the same environment, different individuals exhibit unique behavioral outputs that distinguish them from each other. While decision-making is typically studied as a discrete outcome in a specific context, it remains unclear how it is performed across developmental timescales, and how innate preferences drive inter-individual differences in long-term decision-making patterns. Here we introduce a novel experimental paradigm and analysis methods, akin to the idea of exploitation vs. exploration, to elucidate the neuronal basis of inter-individual variation in decision-making across the entire developmental trajectory of *C. elegans*.

By simultaneously tracking decision-making patterns of multiple individual worms continuously from hatching to adulthood, in an experimentally controlled environment, we identified distinct temporal patterns and inter-individual behavioural differences in decision-making. When presented with bacterial food patches of varying concentrations in spatially segregated locations, most individual worms made the optimal choice, selecting the higher-concentration food patch. However, decreased contrast between food patches concentration, revealed higher individual variability in the decision being made. In addition, we also found significant temporal variation among individuals in the developmental stage in which decision-making is executed. To study the neurobiological basis of inter-individual variation in decision-making we examined the roles of neuromodulators and other sensory transduction genes. Our results suggest context dependent effect of these underlying neuromodulatory pathways on inter-individual variation in decision making across development. Furthermore, to investigate the basis of inter-individual variation within an isogenic population, we have studied the association between variation in gene expression in specific neurons of individual worms and their individual-specific decision-making patterns.

Our results imply a substantial individual diversity in decision making patterns across development, controlled by internal sensory states and environmental context.

594A FLP-15 functions through the GPCR NPR-3 to regulate local and global search behaviours in *Caenorhabditis elegans*

Umer Saleem Bhat[1], Siju Saleem Surendran[1], Sharanya Saleem H[1], Jun Liu[2,2], Namra Saleem Tasnim[1], Monika Scholz[2], Kavita Saleem Babu[1]1Indian Institute of Science, Bangalore, [2]Max Planck Research Group Neural Information Flow, Max Planck Institute for Neurobiology of Behavior

Foraging is critical for normal life and well-being in animals. *C. elegans* explores the area for food locally with reorientations executed frequently. However, the frequency of reorientations decreases temporally on off-food conditions to ensure global search. We screened through genetic mutants of neuropeptides for defects in local and global search behaviors. Among the screened neuropeptides, we found that neuropeptide FLP-15 regulates the frequency and length of reversals during both local and global search. We further observed that FLP-15, is expressed I2 pharyngeal neuron and functions through the G-protein-coupled receptor, NPR-3, to regulate foraging behaviours. Our results show that the mutants in neuropeptide flp-15 and receptor *npr-3* show a significant decrease in reversal frequency during local search. The reversal frequency, however, does not decrease with time in *flp-15* and *npr-3* mutants, and therefore, in global search, the reversal frequency is significantly higher compared to wild-type animals. To further delve into the site of action, we found that NPR-3 is expressed in a subset of head neurons that largely constitute dopaminergic neurons. Interestingly, we also found that FLP-15, possibly through NPR-3, regulates the amplitude of body bends. Our experiments show that *flp-15* and *npr-3* mutants show a significant increase in the amplitude of body bends during sinusoidal movement when compared to wild-type animals and therefore are unable to explore their surroundings effectively. The increase in amplitude in these mutants concurs with an increased expression of neuropeptide NLP-12, previously implicated in causing increased amplitude of body bends in *C. elegans*.

595A Mechanism of feeding state dependent CO2 chemotaxis plasticity

Akankshya Sahu, Pallab Majee, Nargis Mushtaq, Abhishek Bhattacharya National Center for Biological Sciences - TIFR

Animals can modulate their behavioural responses to external cues depending upon their internal states. Hunger plays a major role in modulating animal's response to various external cues across phyla. One of the best characterized examples of the feeding state dependent behavioral modulation is the reversal of carbon dioxide chemotaxis valence in nematodes, where animals display attraction to CO_2 during the dauer arrest stage and shifting to CO_2 avoidance in the replete condition. It has been shown that both *C. elegans* and parasitic nematodes use conserved sensory neuronal circuit to execute these opposing behaviors towards CO2. However, the neural mechanism that regulates the valance of CO2-chemotaxis based on the internal-state of the animal has not yet been fully understood. Our work has previously showed that a de novo electrical synapse formation in the dauer state between the CO_2-sensing neuron, BAG and a hub interneuron, AIB contributes to the robustness of CO_2-attraction in dauer. However, we have discovered that similar electrical synapses between BAG and AIB also form during L1 diapause stage. This prompted us to test whether nematodes exhibit this reversal of CO_2-chemotaxis during other developmental diapause stages and ultimately to understand the molecular mechanism underlying the CO_2 chemotaxis plasticity.

Our results showed that CO_2 attraction is a common feature of all developmental diapause stages, including L1-diapause, L3-diapause and L4-diapause. Similar to the dauer stage, CO_2 chemotaxis in these developmental diapause stages also require BAG neuron. Remarkably, animals could switch from CO_2 attraction to CO_2 avoidance within as little as 30 minutes upon feeding on OP50, suggesting the process happens without extensive circuit modification. Our data suggests that the insulin/IGF-signaling pathway involving the downstream kinases, including AKT-1, but independent of the most well characterized downstream transcription factor, DAF-16/FOXO, is required for the reversal of CO2-chemotaxis upon feeding. Instead, activation of CREB homolog CRH-1 transcription factor downstream of insulin/IGF-pathway is required for the CO_2 avoidance upon feeding. On the other hand, in the diapause stages ubiquitin proteasomal pathway interact with the insulin/IGF-signaling pathway to generate the CO_2 attraction behaviour. We will also present our data on the circuit where these pathways intersect. Based on these results we propose the idea that CO_2 attraction is an innate behaviour of nematodes which gets actively altered to repulsion upon feeding after each developmental molting. This work may also enable us to develop deeper understanding of the host-seeking behaviour of infectious nematodes.

596A Development of an invertebrate model for neurotoxicological risk assessments of Electronic cigarettes

Sonam Bhattarai[1], Kevin Noguchi[2], Andrea Scharf[1]1Biological Science, Missouri University of Science and Technology, [2]Department of Psychiatry, Washington University in St. Louis School of Medicine

Electronic cigarette (EC) use is rapidly increasing due to its perception as a healthier alternative to traditional cigarettes. Furthermore, tobacco funded groups and some governments even encourage pregnant smokers to switch to ECs as a harm reduction strategy. While EC aerosols contain less chemicals than traditional cigarettes, they also expose the user to other potential toxins such as propylene glycol/glycerin (PG/VG) and flavor additives whose safety is untested. In particular, PG can be highly neurotoxic in a dose-dependent manner in the developing mouse brain and produces a pattern of degeneration strikingly similar to NMDA antagonists and/or GABA agonists (NAGAs) like ethanol. Unfortunately, current methods rely heavily on low-throughput and expensive rodent models. Here, our research aims to use *Caenorhabditis elegans*, an *in-vivo* model for neurotoxicological risk assessment of developing brains. This invertebrate offers feasibility advantages over rodents due to its small, well-studied neural circuits. Under a controlled liquid exposure model, we will expose synchronized immature embryos of *C. elegans* (L1) alongside *E. coli* (OP50) to propylene glycol/glycerin (PG/VG) and other chemicals commonly used in industrial solvents and consumer products. Following 48-hours of exposure, they will be transferred to NGM plates to mature into adults. These animals will then be analyzed for neuronal behavior defects that can potentially predict neuropathy in mice and human brains. Here, we will show our first results and discuss our next steps to develop *C. elegans* as a new model for early life neuropathy.

597A TRP-2 mediates proprioceptive ensing by *C. elegans* PDE neurons

Animesh Biswas[1], Hongfei Ji[2], Christopher Fang Yen[11]Department of Biomedical Engineering, College of Engineering, The Ohio State University, [2]Department of Molecular and Cellular Physiology, Stanford University

Proprioception, the ability to sense the body position and movement, is essential for coordinated locomotion. In C. elegans, the proprioceptive PDE neurons sense midbody curvature as part of the compensatory curvature response (CCR), in which anterior body bending amplitude compensates for changes in midbody amplitude. The molecular mechanisms by which PDE senses bending are unknown. A candidate genetic screen identified the TRP-2 ion channel as a key candidate. Mutants defective for *trp-2* show defective CCR behavior, and transgenic rescue of wild-type TRP-2 in PDE neurons rescued the CCR response, confirming its role. Calcium imaging using GCaMP in PDE revealed that PDE neurons fail to respond to midbody curvature in *trp-2* animals, in contrast to wild-type controls. To further dissect the mechanosensory pathway, we induced local perturbations along the body of immobilized worms using a polished glass probe attached to a piezoelectric bender. We found that the midbody ventral perturbation in immobilized worms elicits a calcium response in PDE neurons, and that this response requires *trp-2*. These findings show that TRP-2 is involved in proprioceptive mechanosensation in PDE neurons. However, whether TRP-2 functions as a primary mechanoreceptor or a secondary amplifier remains an open question. Our work highlights TRP-2 as a critical component for the proprioceptive signaling in C. elegans.

598A Uncovering molecular regulators of EtOH-induced behavior deficits in *C. elegans*

Katie Brandel-Ankrapp[1], Rachel N Arey[21]Neuroscience, Baylor College of Medicine, [2]Center for Precision Environmental Health, Baylor College of Medicine

Chronic ethanol (EtOH) use is linked to worsened cognition and has long-lasting effects on cognitive outcomes. In particular, withdrawal from chronic EtOH is associated with persistent memory deficits. One known target of EtOH withdrawal is the a-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptor (AMPAR) EtOH influences this receptor's expression, function, and broad glutamate signaling, particularly during withdrawal (vs. active exposure), implicating its role in regulating neuronal plasticity. EtOH and AMPAR regulation is well-studied in spatial memory and addiction models, and evidence suggests EtOH regulation of AMPARs drive addiction-like behaviors such as EtOH-seeking. However, the exact molecular pathway by which EtOH withdrawal regulates AMPARs in the context of specific associative behaviors is unclear. Due to the complexity of the mammalian brain, it is difficult to unravel the precise mechanism by which EtOH regulates AMPARs in mammals to modify behavior. In *C. elegans*, GLR-1, an AMPAR ortholog, has tightly defined expression in relatively few neurons, has conserved regulatory mechanisms and is linked to molecularly conserved associative behaviors including those disrupted by EtOH. Previous studies demonstrated exposing worms to 400mM EtOH is sufficient to induce 40-50mM internal concentrations that induce acute behavioral phenotypes, whereas 1h of removal is sufficient to decrease internal concentrations to negligible levels and used to model EtOH withdrawal. Using this protocol, I assessed how EtOH affects associative olfactory behavior and GLR-1 dynamics. First, I found that 1h of EtOH removal impairs memory in early adulthood, but 24h EtOH itself does not. Next, I found 1h removal lowers both mRNA and protein levels of *glr-1*/GLR-1. Lastly, preliminary data suggests EtOH and withdrawal modulate GLR-1 transport and localization dynamics in neurons. We are currently probing the molecular pathways that regulate *glr-1* to uncover how EtOH and removal repress *glr-1*/GLR-1 expression. Overall, this work will help inform studies in higher organisms toward the discovery of targeted interventions for EtOH-induced cognitive deficits.

599A Large-scale fluorescence tracking of freely behaving nematodes using megapixel camera arrays

Xiao-Liu Chu[1], Youn Jae Kang[2], Max Hofbauer[3], Weheliye Weheliye[1], Serena Ding[2], André Brown[11]MRC Laboratory of Medical Sciences, [2]MPI of Animal Behavior, [3]Loop Bio

Recordings of freely behaving animals are the foundation of computational ethology and have uses in neuroscience, disease modelling, and drug screening. In the case of the nematode *C. elegans*, most behaviour tracking rigs use brightfield imaging and cannot track worms that aggregate or move in thick bacterial lawns. Here we show that megapixel camera arrays originally designed for brightfield imaging can be adapted for fluorescence imaging. We demonstrate the usefulness of the adapted tracker by tracking worms with fluorescently labelled pharynxes in aggregating groups. To image worms' entire body, we tested several bright GFP lines and show that a worm expressing green fluorescent protein in the pharynx and the rest of the body is bright enough to image in thick bacterial lawns in a 96-well plate. As a proof-of-concept, we track worms on thick lawns of wild microbes co-isolated with *C. elegans* and show that worms behave differently on different strains. Fluorescence imaging of worms over entire multi-well plates extends the applicability of high-throughput behaviour tracking to higher worm densities and more complex environments.

600A DEP-1 is Implicated in Long-Term Associative Memory in *C. elegans*

Noëlle Laura Burri, Attila Stetak, Dominique De Quervain, Andreas Papassotiropoulos, Andreas ArnoldDivision of Molecular Neuroscience,Department of Biomedicine, University of Basel

Protein phosphatases are crucial molecules for regulating cell signalling processes by protein dephosphorylation. For example, the highly conserved receptor-type tyrosine-protein phosphatase DEP-1 (density-enhanced phosphatase-1) plays an important role in vulva development by regulating EGF signalling in *C. elegans* (Berset et al., 2005). Interestingly, a previous transcriptome-wide study found that *dep-1* was upregulated upon aversive olfactory conditioning, suggesting that this gene is also involved in long-term associative memory (LTAM) of *C. elegans* (Freytag et al., 2017). Indeed, we found that a *dep-1* loss-of-function mutant performed significantly worse in the LTAM assay compared to the wild-type control indicating impaired long-term but not short-term memory process. The *dep-1* locus encodes both a transmembrane and a cytoplasmic isoform, giving rise to a receptor-type transmembrane and a cytoplasmic protein, respectively. Expression analysis revealed that the receptor-type DEP-1 was expressed in the developing vulva of L4 animals and is, therefore, most likely responsible for the previously reported vulva-related function. However, the protein encoded by the cytoplasmic isoform, which is regulated by an intronic promoter, was readily detected in the nervous system, also in adult animals. In agreement with this observation, specific inactivation of either of the two isoforms showed that a knockout of the cytoplasmic isoform was sufficient to fully phenocopy the LTAM phenotype previously observed for the full loss-of-function mutant. Overall, these results provide evidence highlighting the specific involvement of the cytoplasmic isoform in the LTAM of *C. elegans*. Currently, we are investigating in more detail how *dep-1* is implicated in the molecular mechanisms of olfactory LTAM. Taken together, this project deepens our understanding of LTAM in *C. elegans* and emphasizes the role of phosphatases such as DEP-1 in memory processes.

601A Predictive modeling to define the locus heterogeneity of tRNA synthetase-related peripheral neuropathy

Allison Cale, Anthony R Antonellis Human Genetics, University of Michigan

Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed, essential enzymes that ligate amino acids to tRNAs in the mitochondria or cytoplasm. Variants in seven genes encoding a cytoplasmic ARS cause dominant axonal peripheral neuropathy, presenting the question: how do variants in cytoplasmic ARSs, which are essential in all tissues, lead to phenotypes restricted to the peripheral nervous system? While defects in protein translation and activation of the integrated stress response have been implicated downstream of certain neuropathy-associated ARS variants, a unifying pathological mechanism that explains the locus heterogeneity has not been identified. Interestingly, all seven neuropathy-associated ARSs function as homodimers and most pathogenic alleles are loss-of-function missense variants; these observations suggest a dominant-negative effect. If a dominant-negative effect is indeed the primary disease mechanism, then certain variants in any homodimeric, cytoplasmic ARS could exert a dominant-negative effect and lead to dominant neuropathy. To test this, we engineered missense mutations in threonyl-tRNA synthetase (*TARS1*), a homodimeric, cytoplasmic ARS not yet implicated in neuropathy. Through humanized yeast complementation and dominant toxicity assays, we identified a *TARS1* missense allele that is loss-of-function, stably expressed, and strongly represses yeast growth when co-expressed with the wild-type allele -- all consistent with a dominant-negative effect. To test the effect of this allele in a multicellular organism with neurons, we introduced the allele (H589A) into the endogenous *C. elegans tars-1* locus using CRISPR-Cas9. We have confirmed through Mendelian segregation analysis that it is a loss-of-function allele in worm, and we will test heterozygous worms, both hermaphrodites and males, in a variety of assays for dominant neurologic and motor behavior phenotypes. We are currently in the process of testing heterozygotes in swim/thrash assays, and our preliminary data suggests that heterozygous worms have decreased motility compared to wild-type worms. We will also perform pharmacological assays that probe neuron function such as the aldicarb assay, and we will augment our phenotypic studies using borrelidin, a chemical inhibitor of *tars-1*. Here, I will present all of our unpublished data from this study in which we aim to elucidate the mechanism by which numerous alleles across seven ARS genes converge on a dominant peripheral neuropathy phenotype.

602A Age-progressive neuronal changes are robustly delayed by caloric restriction through PHA-4/FOXA-mediated regulation of the cell adhesion molecule SAX-7/L1CAM

Yann A Chabi[1,2], Anagha Khandekar[3], Ju-Ling Liu[1], Marin B Pascal[1,2], Lise Rivollet[1,2], Claire Y Bénard[2,4,51]Biological Sciences, Université du Québec à Montréal, [2]Centre d'Excellence en Recherche sur les Maladies Orphelines-Fondation Courtois (CERMO-FC), [3]3.Department of Neurobiology, University of Massachusetts Chan Medical School, [4]Université du Québec à Montréal, [5]Department of Neurobiology, University of Massachusetts Chan Medical School

Cognitive decline during aging is well-known, however the mechanisms by which nervous system dysfunction is triggered during aging remain elusive. Previous studies have reported age-related morphological changes in a subset of *C. elegans* neurons. Here, we have expanded this analysis with a systematic survey of age-related neuronal changes in wild-type animals and we find that during aging, age-progressive alterations arise neuron-type specifically. Notably, we find that structural neuronal changes are uncoupled from lifespan extension per se as an array of long-lived mutants do not delay these neuronal shifts, consistent with findings on healthspan studies (Bansal *et al*, 2015). However, *eat-2* mutants -a genetic model of caloric restriction- stand out, as their age-related neuronal changes are markedly delayed and reduced compared to wild-type counterparts. We delved into the underlying molecular pathways and show that the transcription factor PHA-4/FOXA, which is necessary for the longevity of *eat-2* mutants, is required for the neuronal protective effects of dietary restriction. Interestingly, a potential downstream target of PHA-4 is the gene *sax-7,* which mediates the maintenance of neuronal architecture in *C. elegans* and whose promoter region contains PHA-4 binding sites. SAX-7 is homologous to the vertebrate L1CAM family of cell adhesion molecules, where it also plays post-developmental roles to safeguard cognitive abilities in adult mice. We quantified the levels of expression of *sax-7S* (the key isoform for lifelong neuronal maintenance) using fluorescence and FLIM microscopy, and RT-qPCR, and we find that *sax-7* is upregulated in calorically restricted animals. Moreover, increasing the levels of *sax-7* or of *pha-4* in transgenic animals is sufficient to preserve a youthful neuronal organization in otherwise normally aging animals. In sum, this work uncovers a mechanism where transcriptional regulation by PHA-4/FOXA modulates the expression of cell adhesion molecules such as SAX-7/L1CAM to preserve neuronal circuits during aging. Given the remarkable conservation of genetic and neuronal processes between *C. elegans* and humans, identifying genes that modulate neuronal decline in the worm enhances our understanding of neuronal maintenance and aging. These findings may provide insights into neurodegenerative diseases.

603A Exploring AlphaFold Deep Learning Approach for *Caenorhabditis elegans* neuropeptides GPCRs Deorphanization

Amit Chaudhary, HaoSheng Sun Cell, Developmental and Integrative Biology, The University of Alabama at Birmingham

Neuropeptides are signaling molecules that play fundamental roles in nearly all brain functions. They form extensive ligand-receptor networks through interactions with G protein-coupled receptors (GPCRs). The *Caenorhabditis elegans* genome encodes ~160 neuropeptide-encoding genes and 161 putative neuropeptide GPCRs. Around 60% of these neuropeptide GPCRs are still orphans, meaning that we do not know the neuropeptide ligands for these receptors. Identifying ligands for these orphan GPCRs is a critical challenge in biology, with significant implications for understanding cellular signaling pathways and identifying therapeutic targets. This study employs AlphaFold3, an advanced deep learning-based tool, to systematically investigate the interactions between 344 neuropeptides and 161 GPCRs (55,384 possible interactions). Utilizing AlphaFold3's exceptional accuracy in protein structure prediction and protein-protein interaction modeling, we aim to assess the binding affinities and structural compatibility of neuropeptide-GPCR pairs. Additionally, we will compare our computational predictions with previous experimentally identified neuropeptide-GPCR interactions (Beets *et al*., 2023). Our findings may provide insights into the structural basis of GPCR-neuropeptide interactions, enabling the identification of putative functional partners. This work lays the foundation for experimental validation of computationally predicted pairings, offering a roadmap for understanding GPCR signaling and expanding the pharmacological landscape for diseases associated with these receptors.

604A A scaffold attachment factor PHM-2 regulates synaptic transmission through SLO-2 potassium channel in *C. elegans*

Longgang Niu[1], Sakia Ferdousy[2], Shannon Hanggodo[3], Lishuang Zhu[2], Bojun Chen[21]UConn Health, [2]University of North Texas, [3]Quinnipiac University

Scaffold attachment factor B (SAFB) proteins are evolutionarily conserved DNA/RNA binding proteins that are involved in multiple processes of gene expression. These proteins are broadly expressed with particular high expression observed in the nervous system. However, their physiological roles in neurons are largely unclear. Here we show that PHM-2, the sole SAFB ortholog in *C. elegans*, regulates synaptic transmission at the neuromuscular junctions through an effect on SLO-2 potassium channel. We found that *phm-2* knockout suppresses a sluggish phenotype of worms expressing a hyperactive SLO-2 channel, greatly reduces SLO-2-mediated neuronal whole-cell currents, and enhances neuromuscular synaptic transmission. In addition, we found that PHM-2 genetically interacts with another DNA/RNA binding protein, HRPU-2/hnRNP U, to control SLO-2 expression through a posttranscriptional mechanism. These results reveal a novel function of a SAFB protein in regulating neuronal activity, and may help understand the physiological roles of SAFB proteins in the nervous system of other species.

605A Function of Alternative Splicing in Regulating Olfactory Learning in *C. elegans*

Maoting Chen[1], Min Wu[1], Bina Koterniak[2], John Calarco[2], Yun Zhang[1]1Harvard University, [2]University of Toronto

Learning is an essential function of the nervous system that is regulated by experience-dependent changes. For example, dynamic gene transcriptional levels regulate the plasticity of neuronal function and wiring. Meanwhile, co-transcriptional mechanisms, particularly alternative mRNA splicing (AS) through which a single gene generates multiple different transcripts, are also critical for the complexity of gene expression. Mis-regulation of AS is involved in many neurological diseases that alter learning ability and cause memory loss. Several previous studies have identified individual genes that regulate neural plasticity through alterative splicing. However, the pattern of AS has never been systematically analyzed at the whole genome-level in learning. Here, in this project, we characterize the genome-level experience-dependent AS globally in the entire nervous system during learning, and investigate the mechanisms of specific AS events in learning regulation using *Caenorhabditis elegans* .

In an aversive olfactory learning paradigm, *C. elegans* learns to reduce its preference for the odorants of a pathogenic bacteria *Pseudomonas aeruginosa* PA14 after training on it for 4-6 hours. Multiple neuronal cell types have been shown to regulate this form of olfactory learning. To systematically investigate AS in learning, we performed RNA-sequencing of ribosome-associated mRNAs from the whole nervous system of naive and trained animals, as well as animals that were mocked trained in parallel. Differential expression (DE) and differential AS (DAS) analysis on the RNA-seq datasets showed that both DE and DAS represented the training experience with PA14. Gene ontology (GO) analysis and protein-protein interaction (PPI) analysis on the learning-associated DE and DAS genes suggested that learning induced gene dynamics change at both transcription and AS levels, with each operating on distinct sets of genes. Learning-associated DE genes are enriched in immune and defense response, while learning-associated DAS genes are enriched in canonical neural signaling pathways. With individual DAS genes showing the most significant learning-induced AS change determined, we confirmed several DAS genes involved in the olfactory learning behavior. One of the genes *twnk-1*, encoding a mitochondrial DNA helicase, exhibited significant AS change and regulated neuronal activities during learning. The two related isoforms may contribute to learning with different functions through their alternatively-spliced N-terminals and different neuronal expressions combinatorially.

606A Contact-mediated Mate Recognition Evokes A Persistent Behavioral State via Glutamatergic and Neuropeptide Signaling in *Caenorhabditis elegans* Males

Yin-Chen Lin, Yu-Han Chen, Kai-An You, Tse-Yu Chen, Jia-Bin Yang, Chun-Hao Chen Institute of Molecular and Cellular Biology

Animals respond to internal needs and environmental cues by adopting distinct behavioral states that are timely, scalable, and persistent. Previous studies indicate that *C. elegans* males exhibit a persistent local search state upon contact-mediated mate recognition, but the underlying circuit mechanisms remain unclear. Here, we show that brief mate contact induces a local search state that persistently restricts male locomotion. This behavioral state requires male-specific Ray type B (RnB) sensory neurons, while Ray type A (RnA) neurons play a modulatory role. Optogenetic activation of RnBs triggers a persistent local search behavior resembling that induced by mate contact. RnBs transmit signals via chemical synapses, particularly through glutamatergic pathways. Silencing glutamatergic LUA neurons using chemogenetics abolishes the behavioral transition but does not affect RnB-stimulated male contact behaviors, suggesting that LUAs integrate contact experiences to sustain the persistent behavioral state. Additionally, two neuropeptides, NLP-13 and NLP-49, are essential for maintaining this state, functioning in LUA and non-RnB neurons, respectively, highlighting a role for neuropeptide signaling in circuit-level information processing. Our study elucidates the neural basis of contact-mediated mate recognition and provides molecular insights into how transient sensory experiences are integrated to modulate behavioral states.

607A Deciphering functions and physiology of different *C. elegans* two-pore domain potassium channels

Li Chen1, Siju Surendran2, Lars Nilsson3, Lidia R Sanchez4,5, Thomas Boulin6, Changchun Chen3, Dominique Glauser2, Isabel Beets1, William Schafer1,41Biology, KU Leuven, 2Biology, University of Fribourg, 3Molecular Biology, Umeå University, 4Neurobiology Division, MRC Laboratory of Molecular Biology, 5Department of Psychiatry, Cambridge University, 6Université Claude Bernard Lyon 1

Two-pore domain potassium (K2P) channels are a family of dimeric, 4-transmembrane potassium channels whose members regulate resting membrane potential and other important physiological processes across a variety of organisms. Caenorhabditis elegans harbors the most abundant and diversified K2P channel family; however, the functions of most K2P channels in C. elegans remain largely unclear. To better elucidate their functions, we characterised the expression patterns of several K2P channels, including twk-18, twk-28, twk-9 and twk-23, using reporter lines and guided by transcriptomic data from CeNGEN. We found that twk-28 only expresses in body wall muscles, as reported previously, while twk-18 expresses in body wall muscles and a broad group of neurons including PVC and head glutamatergic neurons. We also verified the expression of twk-9 in IL1 neurons and twk-23 in OLQ neurons. Next, to understand the biological roles of these channels, we generated loss-of-function mutants using CRISPR Cas9 and phenotyped our mutants in various behavioral paradigms. In locomotion tracking experiments, twk-28 mutants showed significantly flatter wave forms as measured by head and hip curvatures. Moreover, we observed significantly higher speed in response to tapping and unfavorable gas conditions in comparison to wild type worms. Both phenotypes were rescued by restoring TWK-28 in body wall muscles. Conversely, twk-9 mutants exhibited attenuated responses to a tapping stimulus, while having normal body curvatures at basal locomotion. twk-23 mutants also showed abnormal locomotion, with exaggerated head curvature in locomotion tracking assays. Lastly, we have been investigating the role of twk-18 in temperature responses, as TWK-18 channels have been reported previously to be activated by heat in vitro. We found that twk-18 mutants behaved differently in a series of postural and locomotory parameters following challenges with high temperature compared to wild type worms. Taken together, these results indicate that a variety of C. elegans K2P channels show loss-of-function phenotypes, consistent with roles in modulating different behaviors. We plan to carry out further cell-specific rescue and calcium imaging experiments with the goal of unraveling their in vivo functions at the neuronal and circuitry level.

608A Epidermal regulation of synapse formation through EAT-20

Salvatore Cherra, Avanti Sawardekar, Zoe Gonzalez, Eliana Keylon University of Kentucky College of Medicine

Non-neuronal cells, like glia, support the formation and maintenance of synapses throughout the nervous system. Although glia promote or inhibit the formation of synapses through secreted and transmembrane proteins, much less is known about the inhibitory actions of these cells. We previously observed that the epidermis is capable of regulating synaptic connections between motor neurons and the muscle. Therefore, we used the neuromuscular junction to screen for mutant single-pass transmembrane proteins that increase synapse formation. We found that *eat-20* mutants produce more synapses throughout post-embryonic development. We observed that EAT-20 functions in a neuron-specific manner, since we only detected an increase in cholinergic synapses but not GABAergic synapses in *eat-20* mutants. EAT-20 is expressed in multiple tissues; however, its expression in the epidermis, but not neurons, is sufficient to reduce the number of synapses in *eat-20* mutants back to wild type levels. Using an EAT-20::GFP knock-in animal, we found that EAT-20 localizes adjacent to axons and synapses along the nerve cord. We are currently investigating how cholinergic motor neurons respond to EAT-20 and how this signaling pathway reduces the formation of cholinergic neuromuscular junctions.

609A Optimizing *C. elegans* as a NAMs model for advanced neurotoxicity assessment

Sooji Choi, Seonyu Lim, Kyung Won Kim Hallym University

The increasing restrictions on animal testing necessitates the development of alternative methodologies for neurotoxicity assessment. Despite global efforts to advance alternative methodologies, standardized approaches are still limited. This study aims to optimize an *in vivo* neurotoxicity assessment method using *Caenorhabditis elegans (C. elegans)*, enhancing its sensitivity, reproducibility, and reliability as a viable alternative to traditional vertebrate-based models. To achieve this, we systematically evaluate key experimental variables — including culture conditions, exposure methods, and data analysis techniques — to develop a standardized protocol. Well-characterized neurotoxic chemicals are used to refine experimental parameters, followed by additional test compounds to validate the method's predictive accuracy. Neurotoxicity assessment in this study is divided into six key aspects: (1) Oxidative stress evaluation – Measuring reactive oxygen species (ROS) levels and antioxidant enzyme activities; (2) Neuroanatomical toxicity – Quantifying structural changes using fluorescence imaging; (3) Neurophysiological toxicity – Assessing neuronal activity and neurotransmitter signaling using molecular profiling with fluorescent reporter genes; (4) Cellular organelle dysfunction – Evaluating mitochondrial, endoplasmic reticulum, and lysosomal activity in relation to neuronal impairment; (5) Locomotion assessment – Measuring movement speed, body bends, and paralysis occurrence using automated tracking systems; and (6) Behavioral analysis – Examining chemosensory and mechanosensory responses to assess sensory and cognitive function. By integrating these multiple neurotoxicity indicators across neurodevelopmental and neurodegenerative aspects, this study aims to establish a highly reproducible, standardized protocol to ensure inter-laboratory reliability. Ultimately, the optimized *C. elegans* model will aid in establishing internationally recognized new approach methodologies (NAMs) for neurotoxicity assessment.

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610A Investigating CED-10's Role in Synaptic Remodeling and Memory Formation in *C. elegans*

Betsy Chow[1,2], Evangeline Chein[1], Angel Garcia[3], Rashmi Chandra[3], Kateryna Tokalenko[4], Vanessa Garcia[4], Hazel Guillen[4], Emma Odisho[4], Emily Soohoo[4], Andrew Bykov[4], Miri Van Hoven[4], Noelle L'Etoile[11]Cell and Tissue Biology, University of California, San Francisco, [2]San Francisco State University, [3]University of California, San Francisco, [4]San José State University

How memory is consolidated during sleep remains a major question in neuroscience. Although neurons were previously considered the primary player in storage for memory, recent findings suggest that Glial phagocytosis engulfing parts of neurons can contribute to this memory storage process, therefore we have to consider Glia. In mammals, astrocytes and microglia have been shown to engulf synapses and in *C. elegans*, the Singhvi lab showed that the AMsh glia prune AFD sensory endings. The AMsh do so in a CED-10 dependent phagocytosis pathway. Whether glia prune synapses in C elegans is not known. Earlier studies in *C. elegans* indicated that sleep after olfactory training is required for synaptic pruning between AWC and AIY neurons and long-term memory consolidation (Chandra et al., 2023). However, the molecular mechanisms through which sleep enables synaptic remodeling are unknown. To close this knowledge gap, we chose to examine CED-10, a key phagocytosis regulator for its role in sleep-dependent memory consolidation. We found that CED-10 mutants failed to consolidate olfactory memory. Further, in collaboration with the Van Hoven lab, we preliminarily found that in these mutants, synaptic downscaling does not occur after training. Next, we plan to determine whether CED-10 is required for sleep. If CED-10 is required for sleep, ced-10 mutant animals would fail to sleep after training and this would indicate that CED-10 and phagocytosis is required for sleep. This would be an interesting finding and could reflect developmental defects in the sleep promoting neuron ALA or another more mysterious role in sleep. If CED-10 is not required, ced-10 mutants would sleep after training as wild-types do. That would indicate that phagocytosis is required for learning independently from sleep. Each finding would give us a deeper understanding of how phagocytosis is used in memory consolidation after training.

611A Developmental plasticity enables flexible feeding and foraging strategies through distinct behavioral state profiles in *Pristionchus pacificus*

Lewis A Cockram[1], Güniz G Eren[1], Monika Scholtz[2], James W Lightfoot[11]Max Planck Research Group Genetics of Behavior, Max Planck Institute for Neurobiology of Behavior – caesar, [2]Max Planck Research Group Neural Information Flow, Max Planck Institute for Neurobiology of Behavior – caesar

Phenotypic plasticity describes the capacity of an organism of a single genotype to express variable traits in response to differing environments. Such traits can encompass morphological, physiological and behavioral modifications allowing organisms to implement different survival strategies. *Pristionchus pacificus*, a nematode of the family Diplogastridae, exhibits a striking and discrete mouth polyphenism. Adults either present with a wide, shallow mouth bearing two claw-like teeth (eurystomatous) or a narrow and elongated mouth cavity with a single reduced flint-like dorsal tooth (stenostomatous). This polyphenism is coupled to different feeding strategies. Stenostomatous animals are strict bacterivores whereas eurystomatous animals can supplement their diet by killing and consuming larval nematodes. Crucially, while the gene regulatory network determining mouth morph fate is well described, mechanisms coordinating the morphology with the corresponding behaviors remain unclear. Here, using a recently developed automated behavioral tracking tool and machine learning model to identify behavioral states, we investigated the coupling of the *P. pacificus* mouth morphology to specific behavioral profiles. We find that eurystomatous and stenostomatous animals display distinct behavioral repertoires in the presence of either bacteria or potential nematode prey. These include altered locomotor activities, characterized by differing dwelling and roaming bouts, and changes to predation-associated behavioral states. To explore morphotype-behavioral associations further we also examined mutants which fail to develop the discrete mouth morphs and instead form intermediate phenotypes. These include *Ppa-mdt-15.1*, *Ppa-spr-5* and *Ppa-nhr-1*. We also investigated the importance of the teeth-like denticles themselves using the toothless *Ppa-chs-2* mutants. Our results reveal mouth-form specific behavioural repertoires consisting of distinct locomotion and feeding states which may reflect their specialised foraging strategies and ecological roles and furthermore elucidate the relationship between mouth morphology and behaviour.

612A Title: Temperature-Dependent Neuroprotection in the MEC-4d Model: Who is the target?

Andrea Calixto[1], Juan pablo Castillo[1], victoria c collio[21]Universidad de Valparaíso, [2]science faculty, Universidad de Valparaíso

Mechanotransduction underlies the senses of touch, balance, proprioception, and hearing. Mechanosensory channels have been identified in bacteria, yeast, insects, and vertebrates, spanning multiple superfamilies. In *C. elegans*, MEC-4, a DEG/ENaC family protein, serves as the pore-forming subunit of the mechanosensory channel in touch receptor neurons (TRNs). A gain-of-function mutation (A713V) in MEC-4, located near transmembrane segment 2 (*mec-4d*), results in a constitutively open channel, leading to unregulated Na+ and Ca+2 influx and subsequent degeneration of TRNs. In this study, we found that while TRNs degenerate almost completely within 72 hours post-hatching at 15°C and 20°C, this degeneration is halted at 25°C, with 86% of axons remaining functional 96 hours post-hatching. These findings suggest that *mec-4d* channel activity may be temperature-sensitive. We hypothesize that MEC-4d itself is a temperature-sensitive protein.

To distinguish *mec-4d*-specific effects from general neuroprotective processes, we examined the role of DLK-1, a key regulator of axonal regeneration that is functionally unrelated to MEC-4. We conducted time-course neuroprotection experiments in *mec-4d* and *mec-4d*; *dlk-1* animals at 15°C, 20°C, and 25°C. As expected after 48 hours post-hatching, at 15°C and 20°C, there was no significant difference in axonal protection between the two backgrounds, with 0% and 0% wild-type axons at 15°C, and 11,1% and 0% wild-type axons at 20°C, respectively. However, at 25°C, while *mec-4d* animals exhibited 84,7% and 94% functional axons with wild-type morphology at 48 and 72-hour timepoints, *dlk-1; mec-4d* animals displayed no wild-type axons at any timepoint. Instead, although the axonal length was comparable to wild-type, the axons exhibited extensive ectopic neurite defects, which make up 82% at 48 hours at 25°C. This suggests that the protective effect of high temperature, which allows axonal regrowth, is *DLK-1* independent—supporting normal axon growth but leading to neurite defects.

Additionally, RNAi-mediated knockdown of *mec-6*, a protein required for MEC-4 trafficking, further prevented degeneration at 20°C, indicating that *mec-6* suppression promotes axonal recovery. However, at 25°C, *mec-6* knockdown had a negligible effect. These results support the hypothesis that *mec-4d* is a temperature-sensitive molecule, as neither DLK-1 nor MEC-6 proteins are needed for the remarkable extent of neuroprotection observed at high temperatures.

613A Examining the impact of receptor affinity on dendrite morphogenesis using synthetic heterodimers

Alec Condon[1], Karly Fear[2], Mohamad Abedi[3], Florian Praetorius[4], David Baker[3], Kang Shen[11]Biology, Stanford, [2]University of Oregon, [3]University of Washington, [4]Institute of Science and Technology Austria

Neuronal dendrites exhibit diverse morphologies tailored to their computational and network roles; however, the developmental mechanisms underlying this diversity remain underexplored. The PVD neuron is a well-established model for studying dendrite morphogenesis and is known to be dependent on the guidance/growth receptor DMA-1. This study examines how dendrite morphology is regulated by the affinity of the DMA-1 receptor through the replacement of native receptor-ligand interactions with synthetically designed heterodimer peptides using endogenous CRISPR modification. By employing a series of heterodimers with varying affinities, this work demonstrates a trade-off between growth (low affinity) and order (high affinity) in receptor-dependent dendrite elaboration. Future investigations will leverage these heterodimers to decouple protein structure from function, providing deeper insights into the mechanisms of DMA-1 signaling.

614A Overlap between regulation of behavioral responses to alcohol and behavioral state

Andrew G Davies[1], Alan'da Thomas[1], Emily Seay[1], Nathaniel Smith[1], Deepanshi Kumar[1], Tanya Kurnootala[2], Tyler Crossen[1], Jonathan Lindsay[3], Laura D Mathies[1], Jill C Bettinger[1]1Virginia Commonwealth University, [2]Perelman School of Medicine at the University of Pennsylvania, [3]Emory & Henry University

The degree to which a naive individual responds to the acute intoxicating effects of alcohol (level of response) is a predictor of their predisposition to develop alcohol use disorder (AUD) later in life. Genetic variation plays a significant role in modulating this level of response. We are using *C. elegans* to identify and characterize the molecular mechanisms by which alcohol interacts with the nervous system and neuronal mechanisms that modulate these interactions. We are particularly interested in behavioral state as a modulator of acute alcohol effects given the strong co-morbidity of emotional states (e.g. depression and anxiety) with AUD in humans. Previously, we identified a nonsense mutation in the *nep-2* gene in a forward genetic screen for alcohol resistance. *nep-2* encodes a metallo-endopeptidase that appears to act by cleaving peptides, which suggests that the accumulation of one or more peptides in the *nep-2* loss-of-function mutant are acting to counter the effects of alcohol and are producing a resistant phenotype. We used genetic suppression of *nep-2* to identify neuropeptide receptors that may be acting downstream of these accumulating peptides. A loss-of-function mutation in *pdfr-1* produces significant suppression of the *nep-2* alcohol resistance phenotype. Given the role that PDFR-1 plays in modulating behavioral state, in terms of roaming and dwelling state choice, we examined the roaming/dwelling phenotype of the *nep-2* mutant and found that *nep-2* mutants show elevated roaming behavior. These data are consistent with a shared mechanism of roaming/dwelling state choice and regulation of behavioral responses to alcohol. We have examined the roaming/dwelling phenotype of additional mutants that show altered behavioral responses to alcohol to determine the degree of correlation of state choice and alcohol response. Current data suggest that the correlation is not absolute, and, as predicted by the promiscuous pharmacology of alcohol as a drug, that there are likely to be multiple pathways by which alcohol responses can be modulated. Further, we have continued to examine additional neuropeptide receptors for a role downstream of *nep-2*-regulated peptides in modulating alcohol response and have identified a significant number of receptor mutants that produce altered behavioral responses to acute alcohol exposure.

615A EEL-1: a conserved E3 ligase required for sickness sleep in *C. elegans*

Bryan Paul D De Galicia[1], Kerry Lecure[2], Brock Grill[3], Paul Mark Medina[4], David Raizen[2]1UP Manila / UPenn, [2]Perelman School of Medicine, University of Pennsylvania, [3]Seattle Children's Hospital, [4]UP Manila

Increased sleep is commonly observed during sickness. While this sleep is likely beneficial in the acute setting, it may be debilitating if it persists. We are using the nematode *C. elegans* to elucidate mechanisms underlying sickness induced sleep (SIS). Through an unbiased forward genetic screen, we identified the gene *eel-1* as required for SIS. *eel-1* encodes a conserved E3 ubiquitin ligase orthologous to human HUWE1, variants of which are associated with X-linked intellectual disability syndromes. We showed that human HUWE1 can substitute for *C. elegans* EEL-1, demonstrating functional homology of these genes, and justifying the use of the worm to understand mechanism. Using transgenic rescue experiments we found that *eel-1* acts in the nervous system. Genetic epistasis experiments show that *eel-1* likely acts downstream of activation of epidermal growth factor receptor (EGFR) in the sleep neurons ALA and RIS, but upstream of signaling by the ALA-derived neuropeptide FLP-13. We are currently identifying precisely where and when *eel-1* is required for normal SIS behavior.

616A Dopaminergic system modulates neurodegeneration in a *C. elegans* model of Spinal Muscular Atrophy

Giada Onorato[1], Pamela Santonicola[1], Federica La Rocca[1], Federica Cieri[1], Valentina Bassareo[2], Giuseppina Zampi[1], Manolo Carta[2], Ferdinando Di Cunto[3], Elia Di Schiavi[1]1IBBR, CNR, [2]Department of Biomedical Sciences, University of Cagliari, [3]Department of Neuroscience 'Rita Levi Montalcini', University of Torino

Spinal Muscular Atrophy (SMA) is a rare neurodegenerative disease, historically considered as a motoneuron (MN) specific disorder. However, this selectivity is in contrast with the fact that the causative gene, *Smn1,* is a ubiquitous gene with housekeeping functions and, in fact, SMA has been recently re-defined as a multi-system disorder. In iPSCs-derived MNs from SMA patients, in SMA mice and in *C. elegans* null mutants in the ortholog *smn-1*, the dopaminergic pathway resulted to be highly dysregulated at the transcriptional and post-transcriptional level. Taking advantage of multiple *C. elegans* SMA models mutated in *smn-1,* we investigated the unexplored connection between SMA and dopamine (DA) in *C. elegans*. We performed DA quantification by HPLC and revealed a reduction in total DA in SMA models, also detected *in vivo* at intracellular level in dopaminergic neurons through the formaldehyde induced fluorescence assay. The Basal Slowing Response (BSR) in *C. elegans* SMA models was found impaired, suggesting that the reduction of DA causes an alteration in dopaminergic neurons function. We also confirmed a reduction in intracellular DA and in BSR in animals silenced for *smn-1* only in dopaminergic neurons, suggesting a cell-autonomous effect of *smn-1*. *bas-1* is responsible of the conversion of tyrosine to levodopa (L-DOPA) and its expression was found reduced in SMA mutants, possibly accounting for the biochemical and behavioural defects we observed. Accordingly, overexpression of *bas-1* in dopaminergic neurons rescued the behaviour defect. In further confirmation, administration of the DA precursor L-DOPA was able to rescue the reduction in intracellular DA and the behavioural defect. Interestingly, we also found that *bas-1* overexpression rescued SMA-related phenotypes in SMA mutants such as the defect in the MNs viability and in the thrashing locomotion. Taken together our results point out to a dysfunction of the dopaminergic system in SMA that may participate in MNs degeneration.

617A The impact of bacterial diet on chemosensory decision-making in *C. elegans*

Arindam Dutta[1], Piali Sengupta[21]Brandeis University, [2]Biology, Brandeis University

In nature, different bacterial species release different combinations of volatile chemicals. *C. elegans* uses its highly developed chemosensory system to detect these odor blends and generate behavioral responses. However, since individual bacterial species do not live in isolation, *C. elegans* must distinguish and discriminate among multiple bacterial odors to select the appropriate bacterial food source. To address how worms make these behavioural decisions, and how their diet influences these decisions, we grew wild-type *C. elegans* (N2) on *E. coli* OP50 and *Enterobacter hormaechei* CEent1 for multiple generations and examined their responses to the odors of each strain of bacteria. Both OP50- and CEent1-grown worms displayed a similar preference level for OP50 and CEent1 odors, showing a mild preference for OP50 odor and a strong preference for CEent1 odor in the absence of other odor cues. Odor-choice assays, in which worms were given the option of selecting either OP50 or CEent1 odors, revealed that while OP50-fed worms maintained their innate preference for CEent1 odor, CEent1-fed worms strongly decreased their preference for the CEent1 odor. This suggests that the OP50 odor may inhibit the preference of CEent1-fed animals to CEent1 odor, a phenomenon not observed in OP50-fed worms. Behavioral assays with neuron-ablated strains showed that while the preference for CEent1 odor is primarily driven by AWC, other neurons may contribute to the mild behavioral attraction to OP50 odor. A recent study (Chai et al. 2024) identified isoamyl alcohol (IAA) as an abundant chemical in the CEent1 odor blend. We found that CEent1-fed worms exhibited a significantly reduced response to a lower concentration (10-3) of IAA compared to OP50-fed worms. This suggests that AWC-driven odor attraction behavior is distinct between CEent1- and OP50-fed worms. We are currently further examining these behaviors and investigating the underlying neuronal and molecular mechanisms.

618A Gap junction-dependent electrical coupling between pharyngeal muscles and marginal cells analyzed by voltage imaging

Nora Isabel Elvers[1], Christin Wirt[1,2], Amelie Bergs[1], Alexander Gottschalk[11]Buchmann Institute for Molecular Life Sciences, Goethe University, [2]Institute of Biochemistry and Molecular Cell Biology, Medical School, RWTH Aachen University

Gap junctions (GJs) are intercellular channels that provide direct cell-to-cell connections, allowing exchange of small molecules, metabolites and the propagation of electrical signals. GJs are formed by two hemichannels (innexons), presumably consisting of eight innexin subunits each. The genome encodes for 25 innexin genes and many cell types express multiple innexins, allowing formation of diverse GJ compositions. To contribute to the understanding of GJ properties and how innexins mediate electrical compartmentalization in the pharynx, genetically encoded voltage indicators (GEVIs) were used. Using GEVI imaging data in pharyngeal muscles (PMs), an optogenetically derived electropharyngeogram (OptoEPG) can be obtained, showing uniform depolarization and revealing spatiotemporally distinct repolarization of anterior and posterior PMs1. Investigation of two innexin mutants, *inx-7* and *inx-6*, revealed that repolarization of the pharynx occurred in a spatiotemporal manner differing from wild type. While *inx-6* mutants repolarized significantly faster in the anterior PMs compared to wild type, *inx-7* mutants showed a shorter delay in repolarization between anterior and posterior PMs.

Another cell type in the pharynx are the marginal cells (MCs), that longitudinally intersperse between the pharyngeal muscles. Because MCs form GJs to neighboring muscle cells2,3, we investigated whether MCs are electrically active and could influence aspects of electrical coupling in the pharynx. Ca2+ imaging using the genetically encoded indicator GCaMP6, revealed Ca2+ fluctuations in MCs during serotonin-stimulated pumping, and voltage imaging of MCs showed electrical activity during pumping. The investigation of *eat-5* mutants showed distinct depolarization patterns of PMs and MCs, which differed from wild type animals. Furthermore, we currently establish dual-color voltage imaging using the two GEVIs, Ace2-2AA-mNeon and QuasAr2, to decipher the temporal differences in de- and repolarization of PMs and MCs. First results indicate that MCs depolarize in synchrony with PMs, but have distinct repolarization patterns differing for the anterior and posterior MCs and indicating complex fluctuations of electrical signals between the two cell types.

1 PMID: 31371514 2 PMID: 19621339 3 PMID: 8805

619A Dopaminergic Neurodegeneration in *C. elegans* is Induced Following Exposure to a Novel Compound Produced by *Streptomyces venezuelae*

Osagie A. Emokpae, Timothy Bushman, Jennifer L. Thies, Lukasz Ciesla, Guy A. Caldwell, Kim A. Caldwell Biological sciences, The University of Alabama at Tuscaloosa

Environmental factors likely interact with gene products to incite neuropathological cascades that lead to neurodegeneration. To model this hypothesized mechanism, our lab previously discovered that exposure to secondary metabolites from the common non-pathogenic soil bacterium, *Streptomyces venezuelae,* causes death of *C. elegans* dopaminergic (DA) neurons and human SH-SY5Y cultured cells. These previous experiments were performed with crude metabolite extract, yet the intricate toxicodynamic mechanisms remained elusive. Recently, we fractionated the major active components of the metabolite extract into two bioactive molecules using HPLC bio-guided fractionation. Since we are still awaiting the NMR data to confirm the chemical structure of the fractions, we refer to them as Peak 1 and Peak 2, with the latter identified as a pure compound. We re-evaluated the effect of Peaks 1 and 2 using several phenotypic assays previously reported for the crude extract. For all assays, comparable phenotypes were observed in *C. elegans* exposed to Peaks 1, 2 and crude extract. As examples, DA neurodegeneration was enhanced following exposure to the *S. ven* metabolic products. Similarly, using *cat-2* mutant worms deficient in DA biosynthesis, we discerned that DA neurodegeneration from exposure to Peak 1, 2, or the crude extract was dependent on the presence of tyrosine hydroxylase. When interrogating Peak 1 and 2 for reactive oxygen species production, we used a 2,7dichloro-fluorescein diacetate assay and saw that both peaks, as well as the crude extract, increased ROS. Moreover, all three *S. ven* metabolic products decrease ATP synthesis in a firefly luciferase ATP assay. Mitochondria dysfunction and oxidative stress are among of the inciting factors that can lead to neurodegeneration. Therefore, we posit that the *Streptomyces venezuelae* Peak 1 and Peak 2 metabolite compounds might serve as environmental neurodegeneration models.

620A A neuroinformatics toolbox for whole-brain calcium imaging analysis in *C. elegans*

Diego Fasoli[1], Yunjie Zhu[1], David C. Hogg[1], Netta Cohen[21]School of Computer Science, University of Leeds, [2]University of Leeds

How brain activity emerges from the concerted interaction of neurons in behaving animals is a major question in neuroscience. Rapid advances in whole brain recordings offer insight into the dynamics of nervous systems, similarities and differences across individuals, as well as unifying principles across species. To date, *C. elegans* has been unique in offering whole-brain recordings at cellular resolution during free behavior, with and without sensory stimulation. To study the whole-brain dynamics in *C. elegans*, we developed a computational toolbox. Our toolbox comprises preprocessing tools (using available GCaMP traces as the input), statistical analysis tools, e.g. to estimate and analyze functional connectomes, and a set of machine-learning models, trained on whole-brain calcium imaging data, and used to predict neuronal activity, from whole-brain data (i.e., from the activity of other neurons, without recourse to behavioral and sensory information external to the brain). Using these tools, we obtained animal-specific statistical models, as well as combined population models, which we use to assess the level of conservation in neuronal functional connectivity across individuals. Our single-animal models revealed that the activity of most neurons can be predicted with high accuracy on time scales previously associated with behavioral states and their transitions. For many neurons, we are able to obtain high accuracy predictions even on faster time scales of 5-10 sec. Using these and population models, we present estimates for information redundancy and distributed computation in the *C. elegans* brain. Finally, we demonstrate that *C. elegans* whole-brain population models can predict the activity of a significant portion of the brain of new animals (outside the training datasets). Our results present a complex picture in which conserved organizational principles are revealed alongside substantial animal individuality. The tools and statistical measures of functional connectivity and inter-neuronal information exchange are already used extensively in the study of the mammalian brain, and will therefore allow for a more unified comparative perspective on whole brain organization and dynamics, with the potential to bridge fundamental mechanistic insights between of meso- and macroscopic scale brain networks in mammalian systems through the insights gained from the *C. elegans* at the microscopic scale.

621A Exposure to imidacloprid and flupyradifurone leads to dopaminergic neurodegeneration and impaired foraging in *Caenorhabditis elegans*

Adam Filipowicz, Keane Bui, Patrick Allard Institute for Society and Genetics, UCLA

Insecticides that target nicotinic acetylcholine receptors (nAChRs), including older neonicotinoids such as imidacloprid and novel butenolides such as flupyradifurone, have been praised for their specific targeting of insect pests; however, mounting evidence suggests that these pesticides may cause significant adverse effects on non-target neurons in non-target species, including bees and humans. Nevertheless, the mechanistic relationship between these pesticides and neurotoxicity across diverse neuronal subtypes is not well understood. We thus tested whether exposure to low doses of either imidacloprid or flupyradifurone cause neurotoxicity across diverse neuronal subtypes in *Caenorhabditis elegans*.

We found that chronic exposure to low doses of either imidacloprid or flupyradifurone caused significant blebbing, an early sign of neurodegeneration, exclusively in dopaminergic neurons in *C. elegans*. This change in neuronal morphology was accompanied by impairment in dopamine-mediated behaviors, namely reduced slowing responses in the presence of food and lowered ability to forage. Cholinergic and serotonergic neurons were not affected, indicating that the degenerative effects of these insecticides are specific to dopaminergic neurons. Neurodegeneration is often accompanied by mitochondrial dysfunction and increased reactive oxygen species (ROS) production. We indeed observed fragmentation of mitochondria in dopaminergic neurons along with elevated ROS upon imidacloprid or flupyradifurone exposure. A ROS-responsive transcription factor, DAF-16, also translocated to the nucleus upon exposure. We were able to reduce the neurodegeneration caused by imidacloprid and flupyradifurone by co-administering the antioxidant N-acetyl cysteine, suggesting that ROS generation is a key mediator of this insecticide-induced dopaminergic neurodegeneration. These findings are significant for two reasons: 1) This is an unexpected off-target effect; *C. elegans* is not a target of these insecticides, and dopaminergic neurons are not thought to be the primary target of these nAChR agonists. These neurons do carry nAChRs, though it is not clear whether any of these are responsible for the toxicity observed here. Other chemical exposures, such as exposure to 6-OHDA, induce degeneration via the dopamine transporter. Future experiments will focus on unraveling the molecular target responsible for the degeneration observed here. 2) Dopaminergic neurodegeneration is a hallmark of Parkinson's disease. To further uncover a potential contribution of imidacloprid and flupyradifurone to Parkinson's disease, we plan on extending our study to an *in vitro* human model.

622A Regulation and consolidation of GPCR signaling through *C. elegans* GOA-1-dependent sleep circuits

Adam Friedberg[1,2], Jackie Cho[1], Manuel Lamela Gomez[1], Anne C. Hart[11]Neuroscience, Brown University, [2]The Warren Alpert Medical School, Brown University

More than one-third of Americans get insufficient sleep; meanwhile, deficient sleep is linked to heart disease, stroke, and increased all-cause mortality. In studies of twins with insomnia, genetics accounted for up to 33% of variance in sleep quality and sleep disturbances, suggesting a strong genetic contribution to inadequate sleep. A gap, however, still exists between identification and investigation of candidate genes and their mechanisms in sleep circuitry. *C. elegans* overcome this hurdle with genetic tractability and conservation of sleep behaviorally *and* molecularly. Classical genetic screens in *C. elegans* have already identified several neurons and multiple genes encoding neuropeptides, G-protein coupled receptors (GPCRs), and G-protein signaling machinery required for sleep, but given the multitude of expression profiles across cell types, few investigations have consolidated molecular findings into *C. elegans* neuroanatomy. Prior sleep studies demonstrate that loss of *goa-1,* encoding the only known G-protein alpha(o) ortholog in *C. elegans*, decreases sleep, and two independent GWAS of human insomnia identify linkage at the locus containing the G-alpha(o) ortholog GNAO1. Here, we leverage genetic epistasis experiments alongside a nonsense-mediated decay system for targeted gene knockdown of *goa-1*. We identify where *goa-1* is required for sleep in *C. elegans* neurocircuitry and identify receptors that are likely to signal through *goa-1* for sleep. By investigating highly conserved sleep-regulatory mechanisms, we strengthen our model's ability to screen for genetic suppressors of common sleep disorders while providing a scalable model to screen pharmacologic agents for improved sleep.

623A Valence of olfactory response in AIY interneuron is determined by integration of multiple sensory inputs

Ryoutaro Higashi, Motonari Kanda, Takeshi Ishihara, Manabi Fujiwara Grad Sch Sci, Kyushu Univ

Olfaction plays a crucial role in many organisms, enabling them to locate food, find mates, and avoid danger. To accurately perceive and appropriately respond to dynamic olfactory environments, the integration of inputs from multiple olfactory neurons is a key function of the nervous systems. However, the underlying principles of this integration and its plasticity remain only partially understood. We analyzed how AIY interneurons in *C. elegans* integrate inputs from multiple upstream olfactory neurons and shape their responses to odorants.

The first-layer interneuron AIY receives direct inputs from the olfactory neurons AWA and AWC, as well as from another sensory neuron, ASE, which also contributes to olfactory perception. When attractive odorants are applied to worms, AIY exhibits an increase in intracellular Ca^{2+} levels, and promotes forward movement playing a crucial role in chemotaxis navigation. We found that in AWA-deficient animals, AIY responds to odor application with a Ca^{2+} decrease, which is the opposite of the wild-type AIY response. These opposing AIY responses are caused by combined inputs from AWC and ASE, because when either AWC or ASE is lost in addition to AWA, AIY responses to the same stimuli return to an almost normal Ca^{2+} increase. Furthermore, if animals lose all three neurons—AWC, ASE, and AWA—AIY responses are completely abolished. Thus, while AIY responses to odor stimuli are driven by inputs from AWC and ASE, the valence of the response (Ca^{2+} increase or decrease) is determined by AWA.

We also found that AWA adaptation, induced by pre-exposure to an AWA-specific odorant, alters AIY responses to other odorants from a Ca^{2+} increase to a Ca^{2+} decrease, mimicking the loss of AWA. This pre-exposure to an AWA-specific odorant reduces chemotaxis to other odorants ("cross-adaptation"). The reduction in chemotaxis is likely due to the inverted responses of AIY, because AIY-ablated animals failed to exhibit cross-adaptation.

Taken together, our results reveal that AIY performs unexpectedly complex computations to shape its responses, wherein one of its inputs determines the valence of the response. This processing allows animals to flexibly adjust their olfactory preferences based on previous experiences with other odorants.

624A Sexually dimorphic functional and structural lateralization in the nervous system of *C. elegans*

Dolev Galski Weizmann institute of science

Bilateral symmetry is an ancient trait found in most multicellular organisms that emerged alongside the development of central nervous systems. Despite the overall symmetry, many species exhibit functional and structural lateralization in some parts of the nervous system, underscoring the important interplay between the two in brain organization and neural circuit function. Here, we show that the connectivity of AWC olfactory neurons is lateralized in males, but in hermaphrodites, various electrical and chemical connections cross the midline to connect left and right sensory and downstream interneurons. We show that males and hermaphrodites exhibit pronounced sexual dimorphism in AWC-dependent sensory perception, integration, and synaptic connectivity, which are important for sex-specific relevant behaviors. In *C. elegans* males, the more lateralized synaptic connectivity enhances mate-finding abilities, while functional differences in odor sensing allow both sexes to perform similarly in chemotaxis tasks. These results demonstrate how the shared and divergent behavioral needs of hermaphrodites and males are met through the interplay of symmetry and lateralization in a sex-shared neural circuit.

625A Characterizing chemosensory neuronal responses through development in both sexes

Sahana Gangadharan[1], Jinmahn Kim[2], Mahdi Torkashvand[2], Maedeh Seyedolmohadesin[2], Sina Rasouli[2], Vivek Venkatachalam[21]Bioengineering, Northeastern University, [2]Physics, Northeastern University

Throughout its life, an animal engages with its environment, prioritizing tasks and adapting to the demands of its surroundings, in an age- and sex-dependent manner. These behavioral differences are often complemented by nervous system changes, across a variety of species. Previous connectomic studies have revealed structural changes in the hermaphrodite nervous system across development (Witvliet *et al.,* 2021) while Haque *et al.* (2024) highlighted how sexual dimorphism arises through distinct developmental gene expression patterns. However, the functional neural dynamics of the developing brain underlying these changes are not well understood.

To address this, we developed 5 microfluidic devices optimized for immobilizing lower larval stage *C. elegans* (L1 - YA) of both sexes to record pan-sensory calcium dynamics (Lin *et al.,* 2021) during exposure to 12 ethologically relevant cues. We studied a diverse set of stimuli that spans concentration-dependent attractive and repulsive gustatory, pheromone, olfactory and nociceptive chemicals.

We observed many distinct patterns of developmentally dynamic responses. (1) Some neurons gradually change sensitivity throughout development in an odor-independent manner (e.g., ASK). (2) Other neurons, however, exhibit odor-specific developmental dynamics (e.g., ASJ gradually increases in sensitivity to isoamyl alcohol, but peaks in responsivity to OP50 at L3). Additional complex responses arose when we compared male and hermaphrodite worms beginning at L3. (1) As animals develop, sensory cells typically show increasing sexual dimorphism in response to odors, though the dimorphism appears at different times for different cells (e.g., ADF responds monomorphically at L3 but dimorphically at L4). (2) Some neurons develop sexually dimorphic responses to different odors at different developmental times (e.g., AWA first responds dimorphically to copper at L3 and to OP50 at L4). (3) Most surprisingly, some neurons show dimorphic responses in larval stages and becomes monomorphic in adults (e.g., ASJ in L3 hermaphrodites responds more strongly to salt than in L3 males, even though ASJ responds monomorphically in young adults). To contextualize these sex-, stage- and odor- specific developmentally dynamic responses behaviorally, we have built microfluidic chips to study chemotaxis towards the stimuli through development.

626A Functional Characterization of the Orphan GPCR F35H10.10 in *Caenorhabditis elegans*

Stephanie Del Pozo[1], Emily Hess[2], Jenis Kim[3], Zayan Sami[4], Jay Garaycochea[31]Computer Science, Goucher College, [2]Chemistry, Goucher College, [3]Biological Sciences, Goucher College, [4]Biochemistry, Goucher College

Neuronal communication is primarily facilitated by neurotransmitter-mediated activation of membrane-bound receptors, including G-protein coupled receptors (GPCRs), which modulate various cellular processes. Despite significant advances in receptor characterization, many GPCRs remain orphaned, lacking identified endogenous ligands and signaling mechanisms. F35H10.10 is an orphan receptor that is implicated to belong to the mgl family of receptors. This study aims to elucidate the function of the orphan receptor F35H10.10 in Caenorhabditis elegans, using behavioral and physiological assays to determine its role in neural signaling and metabolic regulation. Preliminary findings indicate that F35H10.10 knockout (KO) mutants exhibit altered foraging behavior, reduced short reversals, increased food-seeking movement, and decreased pharyngeal pumping, suggesting a role in feeding regulation. Identification of F35H10's downstream signaling pathway can provide insights into neuronal circuit modulation, behavioral regulation and energy homeostasis.

627A Chemosensory activity and sleep regulate sensory synaptogenesis during development in *Caenorhabditis elegans*

Vanessa A Garcia, Hazel Guillen, Maleiyah Harris, Veronica Bi, Cibelle Nassif, Jordan Mitchell, Nebat Ali, Joy Li, Angelina Tang, Vanessa Jimenez, Aruna Varshney, Tanya Ly, Fabiola Briseno, Sukhdeep Kaur, Emily SooHoo, Martina Bremer, Miri VanHoven San Jose State University

Modulation of neural circuits is crucial for perception, learning, memory, and behavior. Defects in neural circuit plasticity have been associated with devastating neurological disorders, such as schizophrenia and dementia. The shaping of synaptic connections by sensory activity during development has been described in many metazoan systems; however, much remains unknown about the mechanisms that underlie this process and whether and how it is impacted by sleep. To investigate these mechanisms, we visualize synapses and examine synaptic changes between PHB chemosensory neurons and AVA interneurons using the fluorescent split GFP-based trans-synaptic marker Neuroligin 1 GFP Reconstitution Across Synaptic Partners (NLG-1 GRASP). Our group discovered a burst in PHB-AVA sensory synaptogenesis during the first larval stage (L1). Synapses are reduced in mutants with PHB cilia defects that impair PHB sensory activity (*che-3*), and in mutants with a loss-of-function mutation in SRB-6, a G-protein coupled receptor that functions in PHB neurons. Additionally, adding chemosensory stimulus to the media significantly increases synaptogenesis, indicating that sensory stimulus can direct the level of synaptogenesis. To understand the role of sleep in the activity-driven synaptogenic burst in L1 animals, we measured PHB-AVA NLG-1 GRASP synaptic intensity every four hours throughout the first larval stage, and immediately before and after the L1 to L2 lethargus, a sleep-like state during the L1 to L2 transition. We observed that PHB-AVA synaptic fluorescence increases significantly at each four-hour interval during the first larval stage, but does not increase significantly during the L1 to L2 lethargus. However, we observed that animals whose sleep was mechanically disrupted during the L1 to L2 lethargus displayed a significant increase in synaptic intensity. We propose that developmentally-timed sleep functions to slow sensory activity-driven synaptogenesis. Our results indicate that the ability of sensory activity and sleep to regulate synaptogenesis is shared even in the most compact nervous systems. These findings also open up this genetically tractable model organism for studies of the molecular mechanisms that underlie these important processes. This work is supported by the NIH (AG091327 and NS087544 to MV and NL).

628A FKH-2 Effects on Mitochondrial Dynamics and Locomotion Patterns in *C. elegans* as a Model for FOXG1 Syndrome Research

Anna Dmytriv[1], Antonio Garnham Leniz[2], Jacob Horn[1], Andrew P. Wojtovich[1], Denise M. Ferkey[21]Department of Anesthesiology and Perioperative Medicine, University of Rochester, [2]Biological Sciences, University at Buffalo

FOXG1 Syndrome is a rare and severe neurodevelopmental disorder caused by de-novo mutations in the *FOXG1* gene, which encodes a forkhead family transcription factor. FOXG1 patients exhibit symptoms such as microcephaly, sleep disturbances, seizures, movement disorders and global developmental delays. These symptoms are caused by improper brain development in the cerebral cortex and corpus callosum. FOXG1 also localizes to the mitochondria where it may promote fission/fusion. Mitochondria carry out essential roles in synaptic activity, neuronal plasticity and brain formation, as these processes are highly energy demanding. *C. elegans fkh-2* encodes a forkhead family transcription factor that shares 82% homology with FOXG1 in its DNA binding domain. We have found that loss of *fkh-2* function results in diminished mitochondrial energy production and reduced locomotion phenotypes that can be rescued by human *FOXG1* cDNA. To develop *C. elegans* as a model for FOXG1 research, our current experiments aim to use CRISPR-Cas9 genome editing to incorporate patient-derived *FOXG1* mutations into *fkh-2*.

629A Striking divergence of RIP interneurons between *C. elegans* and P. pacificus over more than 100 million years of evolution

Luke T Geiger[1], Steven J Cook[1], Ray L Hong[2], Curtis M Loer[3], Oliver Hobert[11]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, [2]Department of Biology, California State University, [3]Department of Biology, University of San Diego

RIP (nerve Ring Interneuron P) provides the sole neuronal connection between the somatic and pharyngeal nervous system – analogous to the vertebrate vagus nerve - in the microbivorous nematode *Caenorhabditis elegans* (*Cel)*. In this species, RIP is a polarized neuron with a dedicated axon and dendrite which consolidates dendritic input from amphid sensory neurons and other ring interneurons. It then transmits this information to targets in the pharynx using acetylcholine and gap junctions. The apparent 'terminal selector' transcription factors which establish and maintain this mature molecular and morphological identity of RIP in *Cel* may be *unc-86* and *ttx-1*. Similarly, the homolog of RIP remains the only neuronal somatic-pharyngeal connection in the opportunistically predatory *Pristionchus pacificus* (*Ppa*). Strikingly, however, *Ppa* RIP directs synaptic output onto several amphid neurons and ring interneurons, in addition to the pharynx. *Ppa* RIP also stains positive for serotonin (5-HT) and expresses the 5-HT synthesis pathway enzyme *tph-1*. Because 5-HT signaling and amphid sensory input play a role in the coordination of pharyngeal muscles and feeding in both species, divergence in the molecular and morphological identity of RIP may have enabled new behaviors involving the pharynx in *Ppa*, such as interspecific predation. To characterize the molecular scope and developmental regulation of cross-species differences in RIP, I employed an *in situ* hybridization chain reaction (HCR) optimized for nematodes. HCR revealed *Ppa* RIP retains expression of both *unc-86* and *ttx-1*. Surprisingly, HCR also shows *Ppa* RIP expresses markers of GABAergic identity *unc-25* and *unc-47* instead of the cholinergic marker *unc-17*. *Unc-86* is unlikely to be a terminal selector for *Ppa* RIP development, as HCR in a *Ppa unc-86* null shows no apparent loss of *tph-1, mod-5, cat-1, unc-25,* or *unc-47* expression in this neuron. I am pursuing similar HCR in a *Ppa ttx-1* reduction-of-function mutant, conducting a forward genetic screen for mutants with loss of RIP fluorescence in a *tph-1p::RFP* line, and testing *Ppa cis*-regulatory fragment reporters in *Cel* to further characterize the genetic changes which drive this divergence. Future single-cell RNA sequencing work I am pursuing will provide a comprehensive neuronal gene expression atlas of *Ppa*, which I will use to interrogate which genes are associated with novel RIP synapses onto amphid and ring interneurons in this species. My transcriptomic atlas will likely reveal further scenarios of evolutionary divergence or convergence in RIP and other neurons.

630A Novel roles of UNK-1 and ZK1073.1 in nervous system function

Lexin W Gitler[1,2], Kang Shen[1,3], Callista Yee[1,31]Biology, Stanford University, [2]Northfield Mount Hermon, [3]Howard Hughes Medical Institute

Proper nervous system function is critical for all aspects of our lives. Nervous system dysfunction can significantly hinder activities and shorten one's quality of life. Numerous neurological diseases, such as Charcot Marie Tooth Disease, have been associated with genetic mutations. We sought to characterize the function of UNK-1/Unkempt and ZK1073.1/NDRG2,4, two highly conserved proteins that have been associated with neurological diseases, in C. elegans neurons. Through CRISPR/Cas9 genome editing, we introduced an auxin-inducible degron tag and mNeonGreen cassette into the endogenous unk-1 and ZK1073.1 loci. With spinning disk confocal microscopy, we observed UNK-1 expression in the cytoplasm of many head neurons, including all dopaminergic neurons (CEP, ADE, and PDE). Auxin-induced degradation of UNK-1 (16h treatment at L4) resulted in increased head oscillations, a phenotype typically associated with defects in neurotransmitter synthesis. ZK1073.1 is expressed pan-neuronally and is localized to the membrane. Using the same degradation conditions, loss of ZK1073.1 resulted in defects in locomotion as measured by observing defects in movement in the bacterial lawn and defects with swimming in M9 buffer. Taken together, we have identified novel roles for UNK-1 and ZK1073.1 function in the C. elegans nervous system.

631A Identification of a new odorant that is detected by the AWCOFF neuron in *Caenorhabditis elegans*

Vaughn E. Brown, Ella Bradley, Sokhna B. Lo, Tymmaa A. Asaed, Elizabeth E. Glater Neuroscience, Pomona College

Caenorhabditis elegans is found commonly in compost, rotten fruit, and other environments rich with bacteria, its major food source. *C. elegans* uses olfaction to discriminate among odorants released by its bacterial food. In *C. elegans* specific neurons have been shown to be required for detecting specific odorants. However, the neuronal basis of detection of many odorants is not known. Our goal was to determine which neuron or neurons are responsible for the detection of attractive odorants released by bacteria. We tested several genetic mutants lacking specific neurons in chemotaxis assays. We determined that the AWCOFF neuron is likely required for the chemotaxis to the attractive odorant 1-butanol. 1-butanol is found in in the headspace (volume of air above bacteria) of the following bacterial species: *E. coli* HB101, *Flavobacterium sp*. JUb43, and *Providencia sp*. JUb39, as well as the culture media LB without bacteria (Worthy et al., 2018). We hope that that this finding will facilitate studies of the role of the AWCOFF neuron in odor detection and discrimination.

632A OpenWorm Project updates - towards a biologically constrained computational model of C. elegans locomotion and development of a worm specific Large Language Model

Padraig Gleeson[1], Yasinthan Vickneswaran[1], Adam Ponzi[1], Danial Kusmanov[1], Jan Uher[1], Ankur Sinha[1], Stephen D Larson[2,31]Neuroscience, Physiology and Pharmacology, University College London, [2]OpenWorm Foundation, [3]MetaCell

The OpenWorm project (http://openworm.org) is a global, online collaboration of computational and experimental neuroscientists, software developers and interested volunteers with an ambitious long-term goal: creating a cell-by-cell computer model of the worm *C. elegans* which reproduces the behaviour of the real animal in as much detail as possible. The project takes a unique Open Science approach to development, and provides a community resource which consolidates our anatomical and physiological knowledge of the worm, allowing investigators to examine the mechanistic underpinnings of how behaviour is generated by a complete nervous system. We will provide an update on the overall roadmap of OpenWorm, and highlight two specific subprojects: development of biologically constrained models of worm locomotion, and creation of a Large Language Model (LLM) customised with curated information on worm anatomy and physiology.

OpenWorm contributors have already developed a 3D worm body model (Sibernetic) which incorporates a fluid mechanics simulator for modelling the interactions between the worm body, driven by its musculature, and the external environment. While this can be used for detailed simulations, it is computationally intensive. We have adapted published 2D worm body simulators for use in the project as more efficient alternatives for testing the generation of behaviour in the body by the nervous system. We will also describe biophysically detailed neuronal cell models which have been translated to standardised NeuroML format to ease incorporation into our simulations. Using these cell models, as well as up-to-date multimodal connectivity information from the *C. elegans* Connectome Toolbox, allows the community to create more biologically realistic neuronal circuits for driving the 2D and 3D worm body models (http://openworm.org/simulate).

LLMs hold great promise for facilitating access to huge amounts of scientific literature across multiple domains. We will outline our work to create a corpus of scientific literature and data related to *C. elegans* which can be used to fine-tune LLMs to allow extraction of scientific knowledge related to the worm (based on Retrieval Augmented Generation). An online interface to this is available at https://openworm.ai. We demonstrate how this custom LLM can be used to help validate computational models of worm anatomy, physiology and behaviour.

633A Kin identity in *Pristionchus pacificus* is encoded by the structural and chemical properties of the nematode surface

Desiree L. Goetting[1], Fumie Hiramatsu[1], Veeren Chauhan[2], James W. Lightfoot[11]Genetics of Behavior, Max Planck Institute for Neurobiology of Behavior, [2]Advanced Materials and Healthcare Technologies Division, School of Pharmacy, University of Nottingham

Kin recognition is deeply conserved across phylogeny. Yet, despite its ubiquity, the molecular and neural mechanisms underlying the ability to discriminate kin remain poorly understood. Recently, the predatory nematode *Pristionchus pacificus* has emerged as a powerful new model for investigating the molecular determinants of kin recognition. In *P. pacificus,* a robust kin-signaling mechanism prevents individuals from being cannibalized by closely-related conspecifics. Currently it is known that effective kin signaling requires the small peptide, SELF-1, and that detection of kin is mediated by direct contact between the predator nose-tip and the cuticle of the prey animal. However, the role of *self-1* in generating the putative kin signal, the final localization of this cue, and what role the specific properties of the cuticle play in encoding kin identity remain unknown. Therefore, to overcome these challenges, we are utilizing a combination of genetic methods and surface sensitive mass spectrometry to identify the structural and molecular components which facilitate the kin-discrimination abilities of *P. pacificus*. The nematode cuticle is an apical extracellular matrix lining the body surface composed primarily of a complex 3D assembly of collagens covered by a lipid and carbohydrate-rich outer layer. Using a candidate-based, reverse genetics approach we have targeted distinct classes of putative cuticular collagen genes to investigate the importance of the surface structure for kin-signaling. We identified several cuticular collagen components, including *Ppa-prl-1/sqt-1*, *Ppa-dpy-2* and *Ppa-col-138,* that are required to establish the kin cue. Mutations in some of these collagens result in significantly more severe kin-recognition defects than those previously described in *self-1* mutants, suggesting that additional signals may also contribute to kin determination. Moreover, we recently found that *self-1* and other kin-recognition mutants acquire aberrant surface chemistries including abnormal surface-lipid profiles. Consequently, we are investigating any potential chemical anomalies in these mutants using 3D Orbisims surface-sensitive mass spectrometry. Thus, together these data support a model wherein both structural and surface components are potentially necessary to convey kin-identity information and influence behavior in *P. pacificus*.

634A Computer vision analysis reveals HIF-1 mediated dendrite regeneration after hypoxic insult through F-actin restructuring

Kin Gomez Canales[1], Adriana San Miguel[21]Chemical and Biomolecular Engineering, North Carolina State University, [2]Chemical and Biomolecular engineering, North Carolina State University

HIF-1 is a hypoxia-inducible transcription factor that drives cell adaptation to low oxygen conditions. In mammals, HIF-1 promotes cerebral recovery from ischemia primarily through endothelial pathways that support blood-brain barrier reorganization. However, little is known about the specific mechanisms through which HIF-1 influences neuronal regeneration, particularly in the absence of an endothelial system. In this study, we reveal a molecular mechanism through which HIF-1 protects neurons intracellularly.

We exposed C. elegans to different stressors to assess dendrite degeneration and recovery. We developed a computer vision system to quantify structural changes in the PVD neuron, that are undetectable by traditional image segmentation. The analysis revealed that under 0% oxygen (anoxia), PVD dendrites developed beading, a hallmark of neurodegeneration. Remarkably, when these worms were returned to normoxic conditions for 24 hours, they demonstrated a significant ability to recover. In contrast, worms cultured under 5% oxygen (hypoxia) failed to recover and even showed worsened beading over time.

We hypothesized that HIF-1 activation might be critical for recovery, as its minimally expressed under 5% oxygen culture. To test this, we used Dimethyloxallyl Glycine (DMOG), to chemically activate HIF 1 in the hypoxic group. While recovery in this group was less pronounced than in the anoxic group, it still provided significant protection, suggesting that HIF-1 activation facilitates dendrite recovery from hypoxic stress.

To explore the cellular mechanisms behind this ability to recover, we analyzed the distribution of autophagosomes, mitochondria, and structural proteins such as F-actin and microtubules in the PVD neuron using fluorescence microscopy. Given the highly branched structure of the PVD neuron, we also developed a computer vision system to categorize the branching degree of dendritic segments. This analysis revealed that F-actin was enriched in the 4th-degree dendrites following anoxic culture and DMOG treatment, but not in hypoxic conditions. Given that HIF-1 can regulate F actin polymerization, HIF-1 activation may reorganize F-actin filaments to reinforce dendritic regions more prone to beading, facilitating recovery after the stressor is removed. This study advances our understanding of neuronal recovery by revealing a molecular mechanism through which HIF-1 activation regulates F-actin reorganization.

635A Investigation of a conserved BTB/POZ domain containing protein enriched in dopamine neurons

Alexandro Gonzalez[1], Brian L. Nelms[21]Fisk University, [2]Life and Physical Sciences, Fisk University

The dopaminergic system regulates motor coordination, memory, learning, reward processing, motivation, and mood across animal species. Dysfunction in dopaminergic signaling correlates with debilitating physiological and psychiatric disorders such as Parkinson's, Alzheimer's, Schizophrenia and depression. Understanding the genetic and post-transcriptional dysfunctions of the dopaminergic system is critical for developing pharmacological interventions and preventive therapeutics. Research using the model organism *Caenorhabditis elegans (C. elegans)* has been instrumental in investigating the molecular mechanisms of dopaminergic-mediated locomotion. Similar locomotion impairments are observed in mammals and *C. elegans* upon genetic manipulation of the dopaminergic system. Investigation of the dopaminergic neuron transcriptome in *C. elegans* has unveiled unstudied genes potentially essential for dopaminergic development. A gene we refer to as *ktl-8* shows high expression in the dopaminergic system of *C. elegans* with yet unknown function. Interestingly, structural analysis of KTL-8 and a human ortholog, potassium channel tetramerization domain containing (KCTD#) protein, reveals conservation of the BTB/POZ domain. The BTB/POZ domain is often involved in transcription protein-protein interactions, and in mammals, some BTB/POZ proteins regulate neuronal development and synaptic plasticity. We will analyze deletion mutants of this gene for dopamine-related phenotypes. Understanding the function of *ktl-8* may uncover new molecular targets for locomotion disorders while also providing insight into species-specific adaptations in dopaminergic regulation.

636A Exploring the impact of intestinal infection and immune response on dopaminergic neurodegeneration in Parkinson's disease models

Juan Carlos Gonzalez-Orozco[1,2], Alejandro Aballay[1,21]Department of Genetics, MD Anderson Cancer Center, University of Texas, [2]Department of Microbiology and Molecular Genetics, McGovern Medical School at UTHealth, Houston, TX, United States, 77030.

Neurodegenerative diseases, such as Parkinson's disease (PD), are increasingly linked to immune response and chronic inflammation. Moreover, accumulating evidence indicates that intestinal inflammation caused by infections or microbiota dysbiosis plays a role in PD progression through the gut-brain axis. However, the mechanisms underlying this relationship are not yet fully elucidated. Using *C. elegans* as a model, we investigated how intestinal infection influences dopaminergic neurodegeneration in PD models. Our findings show that infection with *P. aeruginosa*, *S. enterica*, or *E. faecalis* does not induce dopaminergic degeneration in healthy worms but significantly accelerates neurodegeneration in PD models. Because different types of pathogenic bacteria induce the same acceleration effect, it is possible that a generalized molecular response to pathogens, such as the innate immune response, is involved in this deleterious process. Interestingly, healthy worms exposed to *P. aeruginosa* across generations (P0 and F1) result in F2 progeny that exhibit susceptibility to dopaminergic degeneration upon infection. RNAseq analysis of F2 worms from infected lineages reveals persistent upregulation of immune response genes, even in the absence of *P. aeruginosa* exposure, suggesting a long-lasting transcriptional imprint that induces susceptibility to dopaminergic degeneration once the worms are infected. Based on these results, we analyzed several RNAseq datasets obtained from worms exposed to pathogenic bacteria, worms whose progenitors were exposed to pathogenic bacteria, PD *C. elegans* models, and PD human patients with the goal of identifying immune response genes that are commonly regulated in all the mentioned backgrounds, with future studies aiming to validate their role in PD neurodegeneration using *C. elegans* genetic approaches. Our work could provide insights into how host-pathogen interactions and immune responses may exacerbate neurodegenerative processes, potentially revealing novel targets for PD.

637A A non-apoptotic role of EGL-1 and CED-4 to inhibit anterior QL.a and QL.p migration downstream of MAB-5/Hox

Celeste J Gormly[1], Erik Lundquist[21]Molecular Biosciences, University of Kansas, [2]University of Kansas

Neuroblasts QL and QR are born bilaterally in the posterior region of *C. elegans* between the V4 and V5 hypodermal seam cells. QR migrates anteriorly over V4 and QR migrates posteriorly over V5, and both cells divide. EGL-20/Wnt is secreted by muscle cells near the anus where QL daughter cells QL.a/p encounter it. EGL-20 drives the expression of MAB-5/Hox in QL via canonical Wnt signaling. MAB-5 is both necessary and sufficient to drive posterior migration. Before MAB-5 re-programs QL.a to migrate posteriorly, it also prevents the default anterior migration of QL.p. FACS sorting, RNA-seq and differential expression analysis was performed in early L1 Q cells from *mab-5* gain-of-function (gof) and wild-type worms to identify genes acting downstream of MAB-5. *egl-1* was significantly upregulated in the *mab-5* gof. EGL-1 is known as the activator of the programmed cell death pathway, however it and the PCD pathway have been implicated in other developmental events that do not result in cell death such as synaptic elimination (Meng et. al, 2015). We screened members of the PCD pathway for misplaced PQR (QL.ap) neurons. We found that while *egl-1* and *ced-4*/APAF mutants alone did not have PQR migration defects, *ced-3*/Caspase 3 mutants had a significant number of QL neurons that failed to complete migration. *egl-1* and *ced-4* mutants strongly enhanced aberrant anterior PQR migration in *egl-20* and *pop-1*/TCF-LEF hypomorphic mutants and suppressed aberrant posterior AQR (QR.ap) migration in *mab-5 gof* mutants. We also identified FLI-1, a member of the gelsolin family of actin cytoskeleton regulatory proteins as a possible downstream target of the PCD. *fli-1* mutants exhibited significant failures in QL posterior migration and enhanced the aberrant anterior migration in both *egl-20* and *bar-1*/b-catenin hypomorphs. These results suggest that the PCD acts downstream of the Wnt signaling pathway during migration of QL.a and QL.p without inducing cell death, and that there is a more complex interaction with actin cytoskeleton regulatory proteins to prevent anterior migration of QL.a and QL.p. Future studies are aimed at further characterizing interactions within this pathway, and determining the mechanisms the PCD uses to inhibit anterior migration.

638A FLWR-1 may be a stimulatory subunit of the plasma membrane Ca2+ ATPase

Marius Seidenthal[1], Jasmina Redzovic[1], Jana F Liewald[1], Dennis Rentsch[1], Noah Schuh[1], Nils Rosenkranz[1], Stefan Eimer[2], Alexander Gottschalk[11]Buchmann Institute, Goethe University, [2]Goethe University

The Flower protein (FLWR-1 in *C. elegans*) was suggested to couple the fusion of synaptic vesicles (SVs) to their recycling in different model organisms. Flower is supposed to trigger activity-dependent bulk endocytosis by conducting Ca2+ at endocytic sites. However, this mode of action is debated, and Alphafold3 structure predictions of putative FLWR-1 tetramers do not support a channel function. We investigated the role FLWR-1 in neurotransmission and in endocytic processes in coelomocytes. Our results confirm that FLWR-1 is a protein of SVs, but is also located to the plasma membrane, in most or all tissues of *C. elegans*. In neurons / at the neuromuscular junction (NMJ), FLWR-1 facilitates the recycling of SVs. Ultrastructural analysis of synaptic boutons after hyperstimulation revealed an accumulation of large endocytic structures in *flwr-1* mutants. These findings do not support a role of FLWR-1 in the formation of bulk endosomes but rather a function in their breakdown. In line with this, an optogenetic imaging based assay for SV fusion and recycling (pOpsicle) confirms a defect in SV recycling in cholinergic neurons. Unexpectedly, loss of FLWR-1 led to increased neuronal Ca2+ levels in axon terminals during stimulation, particularly in GABAergic motor neurons, causing an excitation-inhibition imbalance. We found that this increased NMJ transmission is caused by deregulation of MCA-3, the nematode orthologue of the plasma membrane Ca2+ ATPase (PMCA). *In vivo* molecular interactions indicated that FLWR-1 may be a positive regulator of the PMCA and might influence its recycling through modification of plasma membrane levels of PI(4,5)P2, possibly through a putative PI(4,5)P2 binding site. The latter is supported by the demonstration that *flwr-1* mutants show reduced levels of PI(4,5)P2 in another endocytic cell type, i.e. coelomocytes, where also the function of MCA-3 is required for high PI(4,5)P2 levels and normal endocytosis. Furthermore, FLWR-1 influences the PM localization of MCA-3 in neurons. We suggest that FLWR-1 is inserted into the PM upon SV fusion, and may thus stimulate the function of MCA-3, thus bringing synaptic Ca2+ levels quickly to resting levels. This function, as well as an influence on PI(4,5)P2, ensures normal and efficient endocytosis and SV recycling.

639A Potassium-selective channelrhodopsins can exert hyper- or depolarizing effects in excitable cells, depending on experimental condition

Christiane Ruse[1], Marius Seidenthal[1], Jana F Liewald[1], Linda Tillert[2], Johannes Vierock[2], Alexander Gottschalk[11]Buchmann Institute, Goethe University, [2]Charité, Berlin

The 'zoo' of optogenetic tools used for the control of excitation and inhibition of muscles and neurons is ever-increasing, but their functionality and applicability can differ between different model organisms. Our work focused on the study and characterization of potassium-selective channelrhodopsins (KCRs) in excitable cells of *Caenorhabditis elegans*. We focused on HcKCR1 and WiChR, which were suggested in previous studies to inhibit excitation through hyperpolarization by inducing potassium efflux. Through body length assays and electrophysiological measurements in body wall muscles of transgenic worms, we found that, upon light-induced stimulation of channel opening, they induce only brief hyperpolarization followed by longer and persistent depolarization. This might be caused by the high conductivity of the channels, causing an initial, intense potassium efflux that mediates macroscopic changes in the ionic gradient across the membrane. This is followed by sodium influx through the channels as a secondary effect. To demonstrate this, we are currently conducting ion exchange experiments. Interestingly, we found that lower expression levels, decreased light intensities, wavelengths that differ from the absorption maximum and pulsed instead of continuous illumination, all of which would be expected to minimize the stimulation and thus the membrane conductance mediated by the light-gated channels, increase the hyperpolarizing ability and prevent or decrease the intensity of the subsequent depolarization.

In collaboration with Dr. Shiqiang Gao (University Würzburg, Germany), we are testing HcKCR1 mutants that were found to reduce the depolarizing tendency of the channel in *Drosophila*. This mutation also appears to improve the hyperpolarizing abilities in *C. elegans* in initial experiments.

Establishing KCRs provides an important addition to the rhodopsin-based optogenetic toolbox, which until recently included channels and pumps for Na+, H+, Cl-, and Ca2+, but not K+. This finally allows direct access to mediating fluxes of potassium, the main ion used by cells for repolarization and for defining their resting membrane potential.

640A Synthetic neurobiology in *C. elegans*: engineering permanent ligand-inducible genetic switches to control behavior.

Alessandro Groaz, Paul W. Sternberg BBE, California Institute of Technology

Current synthetic biology efforts have primarily focused on unicellular organisms, such as bacteria, yeast, and isolated mammalian cells. These studies have developed a substantial array of tools and principles for engineering genetic circuits of arbitrary complexity. However, expanding synthetic biology research to multicellular organisms is crucial for applications beyond the laboratory, including ecology, biocontrol, bioremediation, environmental engineering, and tissue-based biosensing. We aim to extend synthetic biology efforts to multicellular organisms by pioneering novel neuro-engineering approaches to control animal behavior in the nematode *C. elegans.*

We are developing a modular toolset to program behaviors in *C. elegans* by inducing stable gene expression changes in response to environmental stimuli. Our approach involves establishing a versatile input module that utilizes G protein-coupled receptor (GPCR) activation to drive gene expression changes. To this end, we are adapting the Tango system, which exploits the recruitment of arrestin to a GPCR upon ligand binding to promote the cleavage of a transcription factor from the receptor, causing the expression of a gene of interest. In our system, Tango activation will drive the expression of ΦC31 integrase, enabling a permanent genetic switch via recombination. We aim to validate our approach by programming a sequential chemotactic behavior by irreversibly switching the expression of two receptors in AWA sensory neurons mediating chemoattractant responses.

Additionally, we aim to create novel functionality in *C. elegans* by decorating the cuticle with proteins of specific functions, starting with calcium indicators and lanthanide-binding proteins.

641A Multiple secondary messenger pathways compose the preconditioning signal and modulate neuroregeneration via CREB

Noa W.F. Grooms[1], Tina Thuy N. Nguyen Hoang[1], Emma C. Nace[2], Samuel H. Chung[1]1Bioengineering, Northeastern University, [2]Biology, Northeastern University

In mammalian lesion conditioning, a peripheral axon injury enhances central axon regeneration by driving broad, well-described transcriptional changes in regeneration associated genes (RAGs) that confer regenerative capacity. However, the "preconditioning" signal initiating these changes remains unclear. DLK (*dlk-1)* has long been established as the primary mediator of conventional (one lesion) regeneration. We recently demonstrated that the transcription factor cAMP response element binding protein CREB (*crh-1*) is the primary driver of conditioning. In seven neuron types, loss of CREB eliminates conditioned (two lesion) regeneration. Removing CREB significantly reduces changes in RAG transcription, indicating CREB mediates transcriptional changes required for conditioning.

We previously established cGMP and Ca2+/activity inhibit and cAMP activates conditioning in the ASJ sensory neuron. We genetically and pharmacologically targeted the three pathways and assessed regeneration with and without functional CREB. We chronically or acutely treat animals with L-cis-diltiazem or Nemadipine-A to reduce activity of cyclic nucleotide gated channels or L-type voltage-gated Ca2+ channels, respectively. Addition of CREB mutation largely abolishes the conditioning effect, indicating that CREB is downstream of each signaling pathway.

In mammals, conditioning is more strongly enhanced by earlier peripheral axon injury (*i.e.*, the preconditioning effect). Interestingly, in mutants with disrupted cGMP and Ca2+ pathways there is very strong regeneration, well beyond the conditioned regeneration we typically note from concomitant lesions. These results suggest that the preconditioning effect may be modulated by the cGMP and Ca2+, but not cAMP, pathways. We are further studying the pathways to more precisely assess how they mechanistically modulate CREB to drive conditioned regeneration. In mammalian processes, these secondary messenger pathways modulate CREB by phosphorylation at distinct sites. Our current work investigates how CREB phosphorylation influences conditioning. A full description of the conditioning mechanism could illuminate a potential therapy for central nervous system and spinal cord injuries that is empowered by the body's intrinsic regenerative capacity.

642A Mechanisms of chemical synapse remodeling in the neural circuits of dauers

Alan Y Gutman-Wei[1], Oliver Hobert[1,2]1Department of Biological Sciences, Columbia University, [2]Howard Hughes Medical Institute

Precise organization of synaptic connectivity between neuron types is key to nervous system function and behavior. Environmental changes can induce hormonal signaling that modifies the synaptic connectivity of neural circuits via mechanisms that are still poorly understood. In response to adverse environmental conditions during development, *C. elegans* enters the dauer diapause state, triggering widespread anatomical, physiological, and behavioral changes. Recent electron microscopy data has shown that chemical synaptic connectivity is dramatically altered in dauer neural circuits compared to other stages. Remodeling of synaptic connectivity in dauers is specific to synapses between certain cell types, and is accompanied by cell-type-specific changes in membrane contact area between neurons, indicating precise and systematic reorganization of the nervous system. Dauer entry is controlled by hormonal signaling that converges on highly conserved transcription factor pathways, DAF-16/FoxO, DAF-12/VDR, and DAF-3/SMAD, which mediate changes in gene expression and behavior in dauers. How changes in gene expression in the dauer stage direct the precise reorganization of nervous system connectivity is still unclear. We are investigating the molecular mechanisms that remodel chemical synaptic connectivity in *C. elegans* dauers by generating split-fluorophore reporters to confirm cell-type-specific changes in synaptic connectivity and membrane contact area that have been observed via electron microscopy. We will then investigate the roles that transcription factors controlling dauer entry, and their downstream cell-adhesion and intercellular signaling molecules play in synaptic remodeling using these reporters. Furthermore, we will correlate anatomical changes in synapses and membrane contacts with the remodeling of functional connectivity between specific neuron types in the dauer nervous system. These results will determine how precise, widespread remodeling of synaptic connectivity in response to environmental stress is orchestrated by hormonal signaling to the nervous system.

643A Combinatorial regulation of a sleep-controlling neuron by LIM-homeodomain transcription factors

Fujia Han, Vishnu Raj, Xinyu Huang, Han Wang Integrative Biology, University of Wisconsin-Madison

Neurodevelopment is the crucial stage that produces and enables the sophisticated nervous system to process external stimuli and respond with animal behaviors. Homeodomain (HD) proteins are a group of transcription factors (TFs) that precisely regulate the transcriptomes of diverse neurons to proceed neuronal development across phylogeny. However, the genetic mechanisms underlying how HD TFs proteins specify neuronal identities and establish unique neuronal functions are still unclear. Here, we report the critical role of LIM-7, a LIM-HD protein, on the development of the ALA neuron, the master neuron for stress-induced sleep (SIS) in *C. elegans*. We identified a viable *lim-7* mutant that is severely defective in SIS. In *lim-7* mutants, the ALA neuron is born with expression of pan-neuronal genes, but fails to express ALA-specific genes, including *flp-24*, an important neuropeptide for SIS. Using a novel cGAL-based gene trap strategy we recently developed, we confirmed that *lim-7* is expressed in the ALA neuron, suggesting that LIM-7 may function cell autonomously. Our *in vivo* structure-functional analysis of the endogenous LIM-7 protein shows that the LIM Interaction Domain (LID) of LIM-7 is essential for ALA development and SIS. CEH-14 is another LIM-HD transcription factor known for regulating the identity and function of the ALA neuron. Our results suggest a model where LIM-7 interacts with CEH-14 through the LID domain, and they cooperatively bind to promoters of specific downstream genes to regulate the development of the ALA neuron. We are testing whether LIM-7 is important for ALA neuron maintenance, and we are identifying specific ALA promoter motifs and investigating how LIM-7 and CEH-14 bind to these motifs to initiate transcription of ALA-specific genes. These results will reveal the mechanisms of how LIM-HD TFs cooperate to regulate the development of the ALA neuron and provide new insights into how the unique identity and function of diverse neurons in the nervous system are precisely specified.

644A Forward genetic screen using transgenic Dendra2::tau *C. elegans* identifies enhancers of tau proteostasis

Marina Han[1], Aleen Saxton[2], Rebecca Kow[2,3], Nicole Liachko[2,3], Brian Kraemer[2,3]1Graduate Program in Neuroscience, University of Washington, [2]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, [3]Division of Gerontology and Geriatric Medicine, University of Washington

Neurons lose integrity of various protein homeostasis mechanisms as a part of aging and disease. Abnormalities in autophagy, proteasome, unfolded protein response, and chaperones have been reported in numerous neurodegenerative diseases, including tauopathies such as Alzheimer's disease and frontotemporal dementia. Previously, we characterized Dendra2::tau Tg, a photoconvertible fluorescent *C. elegans* model of tauopathy expressing wild type human tau to study tau proteostasis. Using this model, we conducted a forward genetic screen to identify genetic enhancers of tau proteostasis by visually screening for increased Dendra2::tau fluorescence. A preliminary screen identified the transcriptional regulator and class B synthetic multi-vulva (SynMuv) gene *lin-61* as a strong enhancer of Dendra2::tau. To preclude *lin-61* as a future hit, we generated Dendra2::tau Tg with the SynMuvA allele *lin-15A*(n767) and excluded potential candidates with SynMuv phenotype while screening. Subsequently, we identified enhancer gene candidates *thoc-2*, *hpo-10*, *rer-1*, *rpn-10,* and *uggt-2*, with the latter three genes related to proteostasis mechanisms. We will validate the gene candidates using independent alleles and characterize behavior, fluorescence level, and protein burden. In conclusion, we have identified potentially novel regulators of tau proteostasis which will be investigated for translational potential.

645A Comparative connectome analysis reveals distinct neuronal network associated with parasitism in the soybean cyst nematode, *Heterodera glycines*

Jaeyeong Han, Abby Thompson, Ellie Conklin, Lav Varshney, Nathan Schroeder University of Illinois at Urbana-Champaign

Plant-parasitic nematodes have evolved modifications to their feeding structures to facilitate plant parasitism. The parasitic nematode *Heterodera glycines* utilizes highly specialized feeding structures, such as a protractible stylet and enlarged glandular cells, to achieve parasitic feeding behavior. These structures are strictly regulated by different muscle classes through the esophageal nervous system during the infection process. This is distinct from free-living nematodes, such as *Caenorhabditis elegans*, which feed via coordinated rhythmic contractions of a continuous series of pharyngeal pump muscles. To investigate the morphological changes and neural circuit rewiring in the esophageal nervous system of *H. glycines*, we reconstructed the esophageal connectome of infective second-stage juvenile *H. glycines* and compared it to existing datasets from late L1 and adult hermaphrodite *C. elegans*. As expected, the *H. glycines* esophagus has undergone significant remodeling, including changes in cell identities and a reduced number of nuclei. We found a significant reduction in the extracellular basal lamina surrounding non-muscular esophageal cells in *H. glycines*, which allows for additional synaptic inputs from the somatic nervous system. In contrast, the *C. elegans* esophagus is isolated by a thick basal lamina that limits its communication with the somatic nervous system to a single class of somatic interneurons. Despite this structural divergence, we observed substantial conservation of neuronal morphologies. Both species have an identical number of neurons, similar positioning of neuronal nuclei, and conserved neuronal process orientations and branching patterns in several homologs. However, comparative connectivity analysis demonstrated distinct rewiring of individual neurons and corresponding changes in neuronal network connectivity. For example, the *H. glycines* I3 homolog neuron has novel postsynaptic partners, particularly including the stylet protractor muscle. These connectivity changes suggest a rewired neuronal network that may have adapted to parasitism in *H. glycines*. This is the first connectome of a parasite and provides insight into the neuronal adaptations associated with parasitic feeding behavior in nematodes, which may contribute to the development of targeted nematode management strategies.

646A Avocado-Derived Fatty Alcohol Induces Reversible Paralysis in *C. elegans* Through a Dopamine-Dependent Mechanism

Raihanah Harion[1], Hala Fahs[2], Fathima Shaffra[1], Yamanappa Hunashal[1], Kristin Gunsalus[1,1,2], Fabio Piano[1,2]1New York University Abu Dhabi, [2]New York University

Paralysis and recovery are fundamental processes in neurobiology, with implications for both anthelmintic drug development and human neurological health. Understanding the mechanisms that regulate these states could provide insight into disorders characterized by impaired motor function, such as Parkinson's disease. We recently published a set of avocado-derived fatty alcohols/acetates (AFAs) that exhibit nematocidal activity against veterinary parasites *in vitro* and *in vivo* (Fahs et al., *Nat Comms* 2025; doi:10.1038/s41467-024-54965-w). In the present study, we investigate the effects of AFA on *C. elegans*, demonstrating that AFA induces a rapid, dose-dependent, stick-like paralysis that remains consistent for several hours. When maintained for longer periods in the presence of AFA, treated worms gradually regained motility to varying extents depending on AFA dosage. Upon supplementation with a second dose of AFA (without removal of the initial treatment), worms again became paralyzed, suggesting that the initial recovery period we observed is an adaptive response. To elucidate the molecular basis of this phenomenon, we employed click-chemistry to attach a fluorescent moiety to AFA. We then used this fluorescently labeled AFA to visualize AFA uptake in *C. elegans* and found that it accumulates in CEP and ADE dopaminergic neurons, based on colocalization with a GFP reporter driven by the *dat-1* promoter (*dat-1p*::GFP). Given this neuronal localization, we investigated neurotransmitter pathways and found that dopamine signaling is important for recovery. RNAi knockdown of *bas-1* and *cat-2* – key enzymes in dopamine biosynthesis – significantly delayed restoration of motility, suggesting that recovery from AFA-induced paralysis is at least partially mediated by dopaminergic pathways. However, the process underlying the induction of paralysis remains unclear. Future investigations will examine potential mechanisms of initiation and recovery to determine whether, for example, AFAs disrupt neuronal signaling directly, and what metabolic changes may contribute to adaptations that facilitate recovery. We anticipate that these studies will provide new insights into the roles of AFAs in modulating neuronal pathways that control reversible paralysis, with potential implications for a deeper mechanistic understanding of movement disorders and possible treatments in humans.

647A Elucidating the role of neuropeptides in the regulation of amplitude of body bends of *Caenorhabditis elegans*

Sharanya Harish Kumar[1], Umer Saleem Bhat[1], Siju Surendran[2], Kavita Babu[11]Centre for Neuroscience, Indian Institute of Science, Bengaluru, India, [2]Department of Biology, University of Fribourg, Switzerland

C. elegans moves by generating sinusoidal dorso-ventral undulations along its body. Regulation of the amplitude of body bends is essential for *C. elegans* to efficiently explore its environment, forage and escape predators. While the synaptic connections are known for the motor circuits involved in the regulation of the sinusoidal undulations, much is unknown about their functional dynamics.

Neurons can communicate with each other via neuropeptides, in addition to neurotransmitters. Neuropeptides modulate behaviour by binding to G protein-coupled receptors (GPCRs) and causing gene expression changes. They function at a larger spatio-temporal range than neurotransmitters. Some neuropeptides have the capacity to interact with multiple receptors, and conversely, some receptors are promiscuous. Due to the complexity of their actions, it is difficult to study their functions in organisms with intricate nervous systems. *C. elegans* is an excellent model system to study the functions of neuropeptides, especially in the context of locomotion.

We performed a preliminary screen of neuropeptide mutant hermaphrodite *C. elegans* for defects in locomotion. We observed that mutants of an FMRF-amide like neuropeptide, *flp-12*, exhibit an increase in amplitude of body bends. According to the *C. elegans* Neuronal Gene Expression Map & Network (CeNGEN) database, FLP-12 is highly expressed in SMB neurons. SMB neurons have been previously shown to regulate the amplitude of body bends. A recent study that mapped peptide-GPCR interactions has shown that FLP-12 may bind to FRPR-8, DMSR-1, DMSR-7, DMSR-8 and EGL-6. Consistent with this study, we also found that *frpr-8* mutants show an increased amplitude of body bends similar to *flp-12* mutants. These observations lead to the formulation of the hypothesis that FLP-12 is released by SMB neurons and acts through FRPR-8 to control the amplitude of body bends. To test this hypothesis, we will elucidate the neural circuit through which FLP-12 functions to regulate the amplitude of sinusoidal body undulations. The results obtained may provide interesting mechanistic insights into how neuropeptides control behaviour.

648A Examining candidate modifier genes using a *C. elegans* model of SOD1 ALS

Anne C Hart[1], Alex Lin-Moore[1,2], Tanya Sinha[1], Jeremy Lins[1], Jasmine Xi[1], Nicole Dennis Talley[1], Mika Gallati[11]Neuroscience, Brown University, [2]Institute of Science & Technology Austria

Decades of work focused on Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) have led to the identification of numerous disease genes and associated patient alleles that lead to dysfunction, degeneration, and loss of specific classes of neurons. However, available treatments for ALS or FTD do not prevent disease onset, progression, or the eventual devastating outcomes for patients; these diseases are incurable.

Our overall goal is understanding the genetic, molecular, and cellular pathways that cause or modify ALS and/or FTD-associated neurodegeneration. Many critical disease-associated pathways are deeply conserved across the animal kingdom, that laboratory models of disease can be used to understand how patient alleles disrupt function, and that this may lead to the development of more effective therapies. Here, we consider the complex genetics of ALS and FTD. At least 15 “causal” ALS or FTD disease genes have been identified whose mutation clearly leads to disease, but numerous other genes likely exist whose perturbation either causes disease less frequently or alters disease onset or progression. In the studies described here, we use *C. elegans* to examine a short list of candidate genes and determine if their perturbation leads to neurodegeneration or modifies defects in an *in vivo* model of ALS caused by mutation of SOD1. Examining these conserved candidate genes and defining their relevance using *C. elegans* can be a first step toward defining and understanding the role these genes play in neurodegeneration associated with ALS or FTD.

649A A closer look at UNC-33 (CRMP) in neuronal polarity maintenance

Yaren Canten, Liu He, Ines Santos, Martin Harterink Utrecht University

Neurons are highly polarized cells, with morphologically and functionally distinct neurites; axons and dendrites. Differences in microtubule organizations between axons and dendrites allow for selective transport into either neurite to set up neuronal polarity. Previously we identified the UNC-44(Ankyrin)/UNC-119/UNC-33(CRMP) complex to function as a microtubule anchoring complex to maintain this organization, by connecting the microtubules to the submembrane cytoskeleton (He et al., 2020).

UNC-33 has three isoforms: short (UNC-33S), medium (UNC-33M) and long (UNC-33L); all of which share their C-terminus that is thought to bind to microtubules. However only UNC-33L contains an N-terminal extension that connects to the UNC-119/UNC-44, making it essential for microtubule anchoring in neurites. The roles of the short and medium isoforms remain unclear. Since CRMP proteins can form homo- or heterotetramers, it is possible that these UNC-33 isoforms also contribute to microtubule anchoring and neuronal polarity. Here we will present preliminary findings using a functional GFP knock-in line and isoform-specific mutants to explore how UNC-33 isoforms contribute to neuronal polarity.

650A Variation in social behaviors among *Caenorhabditis* nematodes

Dustin Haskell[1], Jhelaine Palo[2], Reina Eugene[2], Michael Hart[21]Genetics, University of Pennsylvania, [2]University of Pennsylvania

The ability to respond to complex stimulus and environmental cues is essential for organisms to survive and reproduce. Responding to a wide range of stimuli requires a neuronal network that can integrate cues and execute behavioral responses. Evolution of behaviors occurs ubiquitously in most established ecological niches, especially among closely related species. To uncover the genetic and neuronal drivers of evolving behaviors, we have taken advantage of the large and ancient divergence in the *Caenorhabditis* clade of nematodes. How do different *Caenorhabditis* nematodes respond to environmental stimuli and are behavioral traits shared or distinct? Here, we assayed behavior of twelve members of the *Caenorhabditis* clade, including members of both the *elegans* and *japonica* supergroup, and the basal taxon *C. monodelphis*. For each species, we analyzed social feeding and bordering behaviors, which are well characterized in *C. elegans.*These behaviors are the functional readout of complex sensory integration of multiple sensory cues including pheromones, touch, oxygen/CO2 concentration, and attractive and noxious stimuli. We hypothesized that the evolutionary divergence between species would correlate to divergence in these behaviors. We observed wide variation in aggregate social feeding and bordering behaviors, but the variation did not correlate with evolutionary relatedness of the species. Mixing sexes had a subtle species-specific impact on social aggregate feeding, where presence of both sexes increased the number of aggregating animals in several species. We also analyzed behaviors with combination of two species in the same assay, which dramatically altered aggregate feeding behavior of *C. elegans,* also in a species-specific manner. This result demonstrates that interspecies interactions and sensory cues can override and modify established behavioral paradigms. Ongoing work includes analyzing behaviors with more ecologically relevant conditions for each species.

651A Patient-specific NFU-1 mutations result in chemosensory dysfunction in a *C. elegans* model of Multiple Mitochondrial Dysfunction Syndrome 1

Jiaxi (Reina) He, Kavya Thaker, Caroline Hitsman, Peter Kropp Kenyon College

Multiple Mitochondrial Dysfunction Syndrome (MMDS1) is a fatal, autosomal-recessive disease in humans caused by mutations in the *NFU1* gene. Within mitochondria, NFU1 transports iron-sulfur clusters to recipient proteins such as members of the lipoic acid synthesis pathway and electron transport chain (ETC). Within 15 months after birth, MMDS1 patients develop symptoms including pulmonary hypertension, neurological regressions, and failure to thrive. Neuromuscular impairments and brain lesions further indicate the necessity of NFU1 function in neurons. To better understand the genotype-phenotype relationship of *NFU1* variants in MMDS1, we used CRISPR/Cas9 to generate orthologous *nfu-1* variants in *C. elegans*. We observe that *C. elegans* with patient-specific *nfu-1* variants are sterile and, surprisingly, tend to avoid their *E.coli* OP50 foodsource. Food avoidance by *C. elegans* may be explained by chemosensory defects, including sensation of volatile compounds (AWA, AWB, AWC neurons), water-soluble compounds (ASE, ASH neurons), or coupled by interneurons (AIB, AIY) that integrate neurosignals and regulate the behavioral response of *C. elegans*. Thus, we hypothesize that patient-specific *nfu-1* variants may differentially affect (1) AWA, AWB, and AWC neurons; (2) ASE and ASH neurons; and (3) AIB and AIY interneurons. We are thus investigating the chemosensory phenotypes of each *NFU-1* variant (Gly147Arg and Gly166Cys) to different stimuli and have found that AWA is adversely affected by both Gly147Arg and Gly166Cys, while AWC is affected only by Gly166Cys. In contrast, AWB shows no functional defects with either *nfu-1* mutation. Chemotaxis assays to assess the functionality of ASE and ASH neurons are ongoing.

652A Investigating the role of IDA-1 in the DAF-7/TGF-B pathway regulation of GLR-1 in *C. elegans*

Taylor Herist, Annette McGehee Suffolk University

In *Caenorhabditis elegans*, neuronal and synaptic function is dependent upon various environmental conditions including temperature, access to food, and overpopulation. The DAF-7 ligand, a part of the DAF-7/TGF-B signaling pathway, is reliant on favorable conditions for proper expression. When mutated, *daf-7* has been shown to increase expression of the AMPA-like glutamate receptor, GLR-1. The mechanism used by the DAF-7/TGF-B pathway to regulate GLR-1 expression is not yet known. One potential regulator is IDA-1, a transmembrane protein found on dense core vesicles (DCVs) that is expressed by the same cells as GLR-1. We investigated the role of IDA-1 in GLR-1 regulation by conducting spontaneous reversal assays and quantitative fluorescence microscopy assays on *ida-1, daf*-7, and *ida-1 daf-7* double mutants. *Daf-7* mutants showed increased rates of spontaneous reversals and increased GLR-1::GFP, while *ida-1* and *ida-1 daf-7* mutants showed no significant difference compared to the wild type. These results suggest a role for IDA-1 in the regulation of GLR-1. We measured levels of IDA-1::GFP using fluorescence microscopy and found that *daf-7* mutants had higher IDA-1::GFP fluorescence than wild type. Together these data suggest a model in which *daf-7* mutants have increased levels of IDA-1, which in turn increases expression of GLR-1. To explore this model, we looked at other genes involved in IDA-1 regulation. PAG-3, a transcriptional repressor, has been previously shown to inhibit IDA-1 expression, as *pag-3* mutants have increased expression of IDA-1. To test the involvement of IDA-1 in GLR-1 regulation, quantitative fluorescence microscopy was conducted on *pag-3* mutants to evaluate levels of GLR-1::GFP. Data indicated increased GLR-1::GFP in *pag-3* mutants compared to wild-type, supporting the idea that increasing IDA-1 increases GLR-1 expression. Assays using *ida-1; pag-3* and *daf-7; pag-3* double mutants are being conducted to further understand the mechanism through which the DAF-7/TGF-B pathway regulates GLR-1.

653A Novel tools for the measurement and manipulation of neuronal lysosomal pH reveals a link between acidification and longevity in *C. elegans*

Molly Hodul, Edwina A Mambou, Aimee W Kao University of California, San Francisco

Lysosomes play a pivotal role in cellular homeostasis by degrading and recycling macromolecules, thus maintaining cellular balance. Their function relies on maintaining a highly acidic internal environment (pH ~4.5) necessary for activating hydrolytic enzymes. Disruption of this acidic pH compromises lysosomal function, contributing to a wide range of diseases, including neurodegenerative disorders, lysosomal storage diseases, and cancer. While lysosomal pH is a critical factor in health and disease, accurately measuring it in specific tissues, especially in long-lived, post-mitotic cells like neurons, remains challenging. Current methods often lack the specificity and temporal resolution needed for *in vivo* studies, prompting the need for improved tools to study lysosomal acidity with precision. To address this, we adapted our genetically encoded fluorescent pH sensor, FIRE-pHLy, for use in *C. elegans*. This enabled neuron-specific, longitudinal measurements of lysosomal pH in a living organism. Using this tool, we discovered that neuronal lysosomal pH increases with age, mirroring findings from intestinal tissues. Notably, animals with lower intrinsic neuronal lysosomal pH at early adulthood exhibited longer lifespans, suggesting that maintaining lysosomal acidity may promote longevity. Analysis of longevity-associated mutants (*daf-2*, *clk-1*, *skn-1*, and *isp-1*) further supported this link, as these mutants displayed reduced lysosomal pH compared to wild-type animals. We next aimed to determine whether direct manipulation of neuronal lysosomal pH could influence lifespan. To achieve this, we developed Acido-pHLy, a novel optogenetic tool that employs lysosomal-targeted Archaerhodopsin-3 (Arch3) to acidify lysosomes upon green light exposure. Acido-pHLy validation confirmed light- and ATR-dependent lysosomal acidification *in vivo*. Lifespan studies revealed that continuous lysosomal acidification in neurons extended lifespan by 1.25-fold, with specificity controls ruling out non-specific effects. Importantly, initiating lysosomal acidification in mid-life also extended lifespan, underscoring its therapeutic potential. Our findings highlight lysosomal pH as a modifiable target for promoting healthy aging. The Acido-pHLy tool offers a powerful approach to dissect the mechanistic links between lysosomal function and age-related decline, presenting new opportunities for therapeutic interventions in neurodegenerative diseases.

654A Neurexin function is dynamic throughout the lifespan depending on foraging behavior in *C. elegans*

Fernanda Holloman[1], Carly Seligman[2], Michael P Hart[31]University of Pennsylvania, [2]University of Pennsylvania, [3]Genetics, University of Pennsylvania

Synaptic adhesion molecules, like neurexins, function in various neurodevelopment aspects required to regulate synaptic connectivity, neuronal circuits, and behavior. Variation in neurexins and related genes increases the risk for neurodevelopmental disorders (NDDs), which are characterized by behavioral changes, including communication, cognition, etc. However, the mechanisms by which neurexins contribute to altered circuits and behavioral changes across adolescence and adulthood are unknown. Furthermore, does the loss of neurexin induce permanent changes to circuit structure and behavior?

We recently reported that losing the singular neurexin (*nrx-1*) reduces group feeding and food deprivation response behavior at day 1 of adulthood. However, are these functions of neurexin maintained across development and adulthood? We assessed both behaviors from larval stage 4 (L4) to day 5 of adulthood. We hypothesized that *nrx-1* would function similarly across the lifespan within each behavioral phenotype. Our results demonstrate that *nrx-1* plays an important role in group feeding behavior during early adulthood (days 1-3) but not during adolescence or mid-adulthood (days 4-5). For food deprivation response behavior, *nrx-1* plays a consistent role during early adulthood. These results indicate that *nrx-1* has a dynamic and specific temporal role in group feeding behavior, with ongoing work analyzing food deprivation response behavior during mid-adulthood to compare *nrx-1* temporal functions across behaviors.

Additionally, we use the auxin-inducible degron (AID) system to temporally and spatially control the endogenous protein expression of NRX-1. Preliminarily, results suggest that somatic *nrx-1* is likely important during development and adulthood to regulate group feeding behavior. Current work is analyzing if neuronal or somatic degradation of *nrx-1* impacts group feeding behavior differently. Additionally, AID allows us to carefully dissect the most critical time point in the lifespan of when NRX-1 is required for behavior. Therefore, I will assess how and if *nrx-1*'s role in behavioral circuit structures is modulated across different time points. Group feeding behavior is driven by the RMG circuit that includes connections to multiple sensory neurons. We have shown NRX-1 decreases the number of synaptic puncta of certain sensory neurons in the RMG circuit in day 1 adult animals so next, I will be measuring synaptic puncta from adolescents to adult animals.

655A *spas-1* is Required for Axon, but not Dendrite, Integrity

Claire Howell, Rachid El Bejjani Davidson College

Microtubule impairments are a major cause of axon degeneration and neuron loss. When a cell's microtubules are damaged, cell structure, transport, and organelle shaping are compromised. The human microtubule severing enzyme, spastin, can be used as a model to study neurodegeneration due to microtubule impairments. Spastin/SPAS-1 regulates transport, cytokinesis, and organelle shaping through microtubule severing. *C. elegans* serves as a strong model organism to study microtubule dynamics and neurodegeneration; the *C. elegans spas-1(ok1608)* strain functionally knocks out *spas-1,* the *C. elegans* ortholog of spastin, via a 627 bp deletion. We used the *C. elegans* TRN and PVD neurons to study axon and dendrite degeneration, respectively. I hypothesized that *spas-1(ok1608) C. elegans* would demonstrate progressive TRN axon and PVD dendrite breakage and degeneration due to microtubule structural defects and impairments in intercellular transport and signaling. Confocal imaging of *spas-1(ok1608);zdIs5* and *zdIs5* control animals revealed that *spas-1(ok1608)* animals display progressive axon degeneration that, depending on the TRN neuron, varies in severity and defect type. In contrast, *spas-1(ok1608);wyIs592* animals showed no significant PVD dendrite abnormalities, suggesting that, in humans, spastin may not be essential for dendrite integrity. These findings establish *C. elegans* TRN mechanosensory neurons as a valuable model for studying microtubule-related axon degeneration and highlight the differential impact of *spas-1* loss on distinct types of neurites.

656A Ensheathment of *C. elegans* touch receptor neuron requires extracellular matrix collagens

Yuxin Hu[1], Matt W. G. Walker[1], eMalick Njie[1], Brian Coblitz[1], Daniel Cabrera[1], Ken C. Q. Nguyen[2], David H. Hall[2], Martin Chalfie[11]Biological Sciences, Columbia University, [2]Neuroscience, Albert Einstein College of Medicine

C. elegans uses its lateral touch receptor neurons (TRNs) to sense gentle touch throughout larval and adult life. At hatching, the TRN processes lie adjacent to the body wall muscle, but in the fourth larval stage, the hypodermis engulfs the cells and separates them from the muscles. Ensheathment is disrupted by mutations in at least twenty-three genes [*atrn-1* (attractin), *crt-1* (calreticulin), *eel-1* (HUWE1-like E3 ubiquitin ligase), *emb-9* (collagen), *ensh-1* (ENSH-1), *ensh-2* (ENSH-2), *him-4* (hemicentin), *let-2* (collagen), *mec-1* (MEC-1), *mec-5* (collagen), *mec-7* (β-tubulin), *mec-12* (α-tubulin), *pat-2* (α-integrin), *pat-3* (β-integrin), *pat-6* (actopaxin), *unc-5* (UNC-5), *unc-6* (netrin), *unc-16* (JIP3), *unc-40* (DCC), *unc-52* (perlecan), *unc-97* (PINCH), *unc-112* (FERMT1), and *vab-10* (plectin)]. Mutant animals exhibit defects in ensheathment (the Ensh phenotype) ranging from minor to complete. Complete prevention of ensheathment occurs in mutants of *him-4*, *mec-1*, *emb-9*, and *let-2* (the last two genes encoding extracellular matrix type IV collagens) and in double mutants containing a *mec-5* mutation and a mutation in one of several genes encoding putative attachment proteins (*crt-1*, *ensh-1*, *unc-52*, and *vab-10*; mutation of these genes on their own yields only partial defects). Because *unc-52* and *vab-10* are expressed in the hypodermis, *ensh-1* and *mec-1* are expressed in TRNs, and the collagen genes *emb-9*, *let-2*, and *mec-5* are expressed in body wall muscles, TRN ensheathment requires all three tissues, as supported by tissue-specific knockout and rescue experiments. An attractive hypothesis for the role of the muscle-derived collagens is that they serve as anchors or platforms adjacent to the body wall muscles, enabling the initiation of ensheathment. In this model, attachment molecules like UNC-52 and VAB-10, along with integrin complexes, may interact with the collagen matrix. Additionally, although some Ensh animals are completely or partially touch insensitive, *emb-9* and *let-2* mutants are not. Thus, adult touch sensitivity does not need TRN ensheathment.

657A Regulation of sleep by the K+ channel EGL-2 in *C. elegans*

Xinyu Huang, Fujia Han, Vishnu Raj, Han Wang University of Wisconsin-Madison

Cellular stress from damaging conditions can induce sleep across animal phyla. In *C. elegans*, Stress-Induced Sleep (SIS) is triggered by SISS-1-mediated activation of the Epidermal Growth Factor Receptor (EGFR) signaling primarily in the ALA neuron, a sleep-promoting neuron. However, the molecular mechanism underlying SIS is not fully understood.

To identify genes involved in SIS, we performed a forward genetic screen to find sleep defective mutants. We have identified *egl-2*, which encodes the sole *C. elegans* homolog of the ether-a-go-go (EAG) potassium channel and belongs to the family of voltage-gated potassium channels. The EGL-2/EAG channel has been shown related to neurodevelopmental disorders and cardiac arrhythmogenic disorders in humans, and regulating egg laying, chemotaxis and male mating in *C. elegans*. Here, we have revealed its role as a novel sleep regulator for SIS.

Our preliminary results showed that *egl-2* functions in the ALA neuron to regulate its neuron activity during sleep. Interestingly, neither a well-characterized *egl-2* gain-of-function allele nor an *egl-2* loss-of-function allele had similar sleep defects observed in the *egl-2* allele we identified. Therefore, we are investigating how this specific allele alters the structures and functions of EGL-2 channel and thus modulates the ALA neuron activity during SIS. We are also characterizing potential interactors and regulators of EGL-2, including UNC-43, the *C. elegans* calcium/calmodulin dependent protein kinase II (CaMKII). The findings will reveal the molecular mechanisms underlying how EAG potassium channels regulate sleep.

658A Inhibition of mitochondrial Complex III in *Caenorhabditis elegans* leads to dopaminergic-specific neurodegeneration

Javier Huayta, Joel N. Meyer Nicholas School of the Environment, Duke University

Environmental factors are important contributors to Parkinson's Disease (PD). Multiple studies have demonstrated a role for chemical exposures, and all these chemicals affect mitochondria. There is strong evidence for association with PD for only a few chemicals, and because few people are exposed to significant amounts of those chemicals, they explain only a small fraction of PD. It is not feasible to test all the chemicals that induce mitochondrial dysfunction. We aim at assessing the mechanisms of toxicity for these chemicals including inhibition of all four electron chain complexes, ATP synthase, and Krebs cycle enzymes; redox cycling; mtDNA damage; and uncoupling of ATP production. We aim to define which forms of mitochondrial dysfunction cause dopaminergic neurodegeneration, as well as whether oxidative stress, and ATP depletion, are required for it. This should help focus the efforts to identify chemicals that could contribute to PD. Here, we report results from inhibition of Complex III.

We used *C. elegans* to evaluate the *in vivo* effects of Complex III inhibitors Antimycin A and Pyraclostrobin -which are environmentally relevant pesticides- on dopaminergic neurodegeneration, ATP levels, and redox state. After developmental exposure, we performed fluorescence microscopy of the neurons located in the head area. These chemicals caused dose-dependent dopaminergic neurodegeneration, but did not cause significant damage in glutamatergic, cholinergic, serotonergic, or GABAergic neurons, or in glial cells. Both Antimycin A and Pyraclostrobin increased the ratio of oxidized to reduced roGFP, a reporter of redox state in dopaminergic neurons. Rescue experiments with an antioxidant (N-acetylcysteine) and a blocker of reactive oxygen species (ROS) specific to Complex III (S3QEL-2) reduced dopaminergic neurodegeneration. In addition, the generation of Complex III specific ROS using the SuperNova construct led to dopaminergic neurodegeneration. These results indicate that neurodegeneration linked to Complex III inhibitors exposure is caused by an increased production of mitochondrial ROS.

Specific inhibition of Complex III using Antimycin A and Pyraclostrobin during larval development of *C. elegans* lead to dopaminergic neurodegeneration. This effect is linked to increased mitochondrial ROS production at Complex III. These results will serve to elucidate the mechanistic aspects of chemical exposures leading to PD, steering future research in vertebrate models.

659B Mating dependent modulation of the egg laying circuit

Melissa Ifidzhen, Kevin Collins University of Miami

Animals exhibit many behaviors that are mutually exclusive. In *C. elegans* hermaphrodites, egg-laying and mating behaviors occur at the same vulval organ, with either eggs being released or the male mating spicules being inserted. As such, these behaviors cannot occur at the same time, suggesting feedback mechanisms drive engagement in egg laying or mating decisions. The vulval cells are innervated by the egg-laying circuit, and Ca2+ imaging has shown a sequential pattern of cell activity drives egg laying. In this circuit, the HSN command neurons release serotonin and NLP-3 neuropeptides which stimulate vulval muscle contractility. The vulval muscles are also innervated by the VC motor neurons which are mechanically activated by contraction and facilitate serotonin-induced egg laying. Vulval opening and egg release mechanically activates the uv1 cells, which release tyramine and neuropeptides to feedback inhibit the HSNs and terminate egg-laying.

How the egg-laying circuit acts during mating is unknown. We performed Ca2+ imaging during mating in wild-type hermaphrodites or sperm-deficient *fog-2* females. We find the HSNs remain inactive throughout mating, consistent with our hypothesis that egg-laying behaviors are inhibited during mating. We find that male mating spicule insertion drives a strong Ca2+ transient in the female uv1 cells, consistent with our prior work showing that uv1 cells are mechanically activated. Genetic loss of uv1-expressed TRPV channels (*ocr-2*, *ocr-4*, and *osm-9*) in hermaphrodites reduces the amplitude of uv1 Ca2+ transients following spicule insertion. We also find TRPV mutant hermaphrodites fail to induce male ejaculation and sperm transfer. Expression of Tetanus Toxin in the uv1 cells to block neurotransmission similarly reduces the onset of sperm transfer, suggesting that activated uv1 cells signal to male cells to drive the onset of ejaculation. We find male sperm transfer drives a strong Ca2+ activity in the VCs, possibly in response to vulval opening during sperm transfer into the uterus. Male mating spicule insertion drives vulval muscle Ca2+ activity which is then suppressed until mating is complete. We hypothesize that inhibitory signaling from uv1 and/or VC cells inhibits vulval muscle Ca2+ activity that might lead to premature cessation of mating. After spicule withdrawal, the vulval muscles show strongly rhythmic Ca2+ transient activity, even in matings with no sperm transfer, suggesting this activity is not a result of uterine stretch. We hypothesize that this rebound in vulval muscle activity is caused instead by cessation of inhibitory uv1 and/or VC signaling as Ca2+ levels in those cells returns to baseline. Together, our results show that cells in the female egg-laying circuit coordinate feedback that drives either egg release or mating behaviors.

660B Dissection of a panel of turning behaviors that achieve directional navigation in chemotaxis using WormTracer to precisely extract worm centerlines

Karin Suwazono, Koyo Kuze, Ukyo T Tazawa, Chung-Kuan Chen, Moon Sun Jang, Hirofumi Kunitomo, Yu Toyoshima, Yuichi Iino Department of Biological Sciences, The University of Tokyo

The behavioral mechanisms underlying chemotaxis have been extensively studied using worm-tracker analyses. Two major strategies are well established: the pirouette mechanism (a form of klinokinesis), in which the frequency of pirouettes (a bout of reversal and turns) is modulated based on the temporal changes in chemical concentration, and the weathervane mechanism (a form of klinotaxis), in which worms gradually curve toward preferred chemical concentrations during forward locomotion. Additionally, previous studies have noted that pirouettes are not only modulated in frequency but also exhibits directionality. Our prior research suggested that the directionality is manifested even in a single turn, such that worms are more likely to be oriented toward the chemical source after omega turns. However, the detailed behavioral patterns remained unclear.

To address this ambiguity, we tracked individual worms during salt chemotaxis and extracted their centerlines throughout the process. Extracting centerlines from worm images is not trivial; when worms adopt curled postures, such as omega turns and delta turns (turns in which the head crosses the body), conventional methods often fail to trace the centerlines accurately. We therefore developed an algorithm called WormTracer. WormTracer introduces a loss function that incorporates the continuity of centerlines (worm postures) over time and determines centerlines by simultaneously optimizing the fit between synthetic worm images and real images across consecutive frames.

In our analyses, we defined a “turn” as a movement in which one part of the body makes contact with another part (which therefore includes conventional omega turns and delta turns). Consecutive turns were called a “turn sequence”. We find that collectively, turn sequences are indeed biased such that turning angles are not randomly determined, but worms regulate their turning angles to orient toward higher salt concentrations after a turn sequence. Further categorization of turn types revealed that each turn type exhibits a unique distribution of turning angles, and directional navigation is achieved through both the selection of specific turn type and fine-tuning of turn angles.

Turning behaviors have been difficult to quantify using conventional methods. By employing single-worm tracking and WormTracer to extract precise centerlines, our study revealed an additional layer of complexity in how worms purposefully navigate chemical gradients.

661B Decoding internal models and dynamics of curiosity using the inverse free energy principle

Muneki Ikeda[1,2], Weiqing Chen[3], Saul Kato[1], Shunji Nakano[4], Honda Naoki[3,5,61]Neurology, University of California San Francisco, [2]General Systems Studies, The University of Tokyo, [3]Theoretical Biology, Kyoto University, [4]Biological Science, Nagoya University, [5]Data-Driven Biology, Hiroshima University, [6]System Biology, Nagoya University

Understanding cognitive processes in speechless animals has been attempted by analyzing their behavior. These attempts are based on the normative hypothesis that animals optimize objective functions such as net reward, economic utility, or environmental information. However, animals do not always exhibit such optimal decision-making. One proposed reason is their limited access to complete and accurate properties of the outer world; instead, animals must rely on internally constructed subjective models derived from partial, noisy observations of the environment. Therefore, to gain insight into their cognitive processes, it is crucial to study the inference of these internal models. The inverse free energy principle (iFEP) is a framework for inferring an agent's internal model by incorporating expected reward and information gain, weighted by a 'curiosity' meta-parameter. This parameter, along with the fine parameters of the internal model, is estimated by maximizing the likelihood of observed behavior—the agent's sensory inputs and actions. The application of iFEP necessitates real-time tracking of both the sensory stimuli experienced by the animal and its corresponding behavioral responses. In this study, we investigated the thermotactic behavior of *C. elegans*, wherein worms navigate a thermal gradient toward their cultivation temperature. Using a multi-worm tracker, we recorded worm trajectories at 12.5 Hz for 1 hour within a 13.6 cm × 9.6 cm field of view, while measuring and estimating the spatial thermal gradient. By applying the iFEP in conjunction with particle filtering and Kalman algorithms, we showed that the worms' sensory observations sufficed to construct an internal thermal gradient model that matched the direction and magnitude of the actual gradient. We further found that the estimated 'curiosity' meta-parameter was initially positive early in the assay but later turned negative, indicating a transition from exploratory information-seeking behavior to the exploitation of reward associated with the cultivation temperature. Our approach provides a framework for decoding the normative cognitive processes underlying animal behavior.

662B High-speed and highly accurate tracking microscopy using motion model estimation for interactively manipulating neuronal activity

Kakeru Imada[1], Takashi Morikura[1], Yuki Tsukada[2], Akira Funahashi[21]Center for Biosciences and Informatics, Keio University, [2]Department of Biosciences and Informatics, Keio University

Optogenetic manipulation of individual neurons in freely moving *C. elegans* is a key technique for studying the function of neural circuits. Real-time tracking under high magnification enables tracking neurons at the cellular level, therefore allowing manipulation at the cellular level. However, the rapid movement of neurons in the microscopic field leads two challenges. One is capturing a target neuron without motion blur, and the other is selecting and tracking the target neuron based on the activity in real-time with pan-neuronal expression of a fluorescent marker. To address these problems, we aim to develop a high-speed and highly accurate tracking microscopy to keep the target neuron for measuring and manipulating the neural activity.

First, we tracked and captured freely moving adult *C. elegans* on an agarose gel using our custom tracking system with microscopy. The system tracks *C. elegans* in the microscopic field of 282 × 211 μm at a loop rate of 167 Hz under high magnification (40× objective). In this system, we detected the head of *C. elegans* using high-speed image analysis by extracting the contour of *C. elegans* from the acquired image. Because the detection algorithm only require the contour information, the computation time is reduced compared to the whole regional image processing. As a result, we achieved a high-speed detection rate of 0.16 ms per frame. We corrected the detection results using Interacting Multiple Model (IMM) filter for improving detection accuracy. The IMM filter integrates multiple locomotion models of *C. elegans* and refines detection results by weighting the prediction of each model. To evaluate the effectiveness, we calculated the Euclidean distance between the detected and actual head positions as detection errors. As a result, the IMM filter reduced the detection error by several percentage points.

This study provides a framework for precise neural activity manipulation in freely moving *C. elegans*. Combining our system with optogenetics enables closed-loop manipulation for interactive experiments. This approach will allow real-time modulation of neural activity in response to observed neuronal dynamics, which will serve as a foundation for understanding behavioral regulation by neural circuits.

663B A forward genetic screen in *Caenorhabditis elegans* identifies *twk-14* as a modulator of α-synuclein-induced neurodegeneration

Joy D Iroegbu, Karolina Willicott, Kim A Caldwell, Guy A Caldwell Department of Biological Sciences, The University of Alabama

We previously reported that the intrinsically disordered protein, a-synuclein (a-syn), a primary pathologic factor in Parkinson's disease (PD), chronically activates the mitochondrial unfolded protein response pathway (UPRmt), resulting in the progressive neurodegeneration of *C. elegans* dopaminergic (DA) neurons. This was mediated through a conserved regulator of the UPRmt, ATFS-1, which activates the transcription of hundreds of nuclear genes that are targeted to the mitochondria in response to mitochondrial stress. In an *atfs-1* loss-of-function *(lf)* background, the burden imposed by an unfettered ATFS-1-dependent transcriptional response is eliminated, resulting in robust neuroprotection from a-syn overexpression. However, the compensatory mechanisms that underlie a-syn neuroprotection are unknown. A forward F3 genetic screen in *atfs-1(gk3094) (lf)* mutant background uncovered a nonsense allele of the *C. elegans twk-14* gene, *twk-14(ba20),* that abolished neuroprotection. The mutation results in a stop codon instead of a tryptophan (Trp358*). TWK-14 is the worm homolog of human KCNK12, a two-pore domain K+ leak channel (K2P) that regulates resting membrane potential. Like KCNK12, *twk-14* expression is limited to neurons. The defined connectome map of *C. elegans* localizes TWK-14 to sensory neurons of the head, in a postsynaptic position within the DA circuitry. We independently confirmed the *twk-14(ba20)* genetic lesion using CRISPR technology. A strain encoding a SNP was created in the endogenous gene product reflective of the Trp358* amino acid nonsense mutation [*twk-14(syb68630*]. Worms expressing this allele was crossed to worms expressing either a-syn or GFP in the DA neurons. Similarly, a CGC *twk-14* allele, *twk-14(tm2522)*, which a 342 bp exon deletion was also crossed to both a-syn and GFP only transgenic worm strains. Both *twk-14* mutations enhanced DA neurodegeneration in the a-syn background but not in the GFP background. Crossing the *twk-14* mutants to the *atfs-1(lf)* allele is ongoing. To date, our screen has uncovered that TWK-14 is protective from the degeneration associated with a-syn expression in *C. elegans* DA neurons.

664B Variation in *C. elegans* social clumping behavior reveals polygenicity of ASD risk genes

Swetha Iyer, Brooke Frohock, Kaelin Rubenzer, Lisa Wang, Jon Pierce University of Texas at Austin

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by atypical social behavior. While >100 ASD risk genes have been identified, how mutations in these genes contribute to ASD-related phenotypes remains poorly understood. Rodent models have facilitated progress with a subset of ASD risk genes but still there are hundreds of genes, yet to studied for their implications in ASD. To accelerate this progress, we are leveraging the rich genetics and quantifiable behaviors of *C. elegans*, which carries orthologs for ~70% of human ASD risk genes.

C. elegans displays a simple form of social behavior called social clumping (de Bono and Bargmann, *Cell* 1998; Portman, *J Neurogen*. 2020). We recently found that wild strains from different habitats around the world exhibit high clumping levels (50-70%), whereas strains carrying predicted deleterious variants in ASD risk orthologs (*nlg-1/NLGN1, gap-2/SYNGAP1, mbk-1/DYRK1A*) show reduced clumping (0-50%). Transforming functional N2 copies of these orthologs significantly boosted clumping. Conversely, mutating genes (*athp-2/BAZ1B* and *mrck-1/DMPK*) associated with hypersociality form of ASD called Williams syndrome (WS) in the low clumping N2 strain, boosted clumping. Collectively, these findings suggest that ASD and WS related genes play a role in modulating social clumping behavior. Our results complement those of Chalasani and Hart labs, which recently found that ASD risk orthologs *nlg-1/NLGN3* and *nrx-1/NRXN1* regulate clumping behavior in *npr-1(loss of function)* background (Cowen et al., *Nat Commun*. 2024). This led us to hypothesize that social clumping behavior can be a useful model to study functions of ASD risk genes.

To independently test this hypothesis, we conducted a genome wide association (GWA) analysis for clumping across wild *C. elegans* strains, predicting that we may identify variants in ASD risk orthologs. We identified two significant quantitative trait loci (QTL) associated with clumping variation. Interestingly, the top QTL contains deleterious variants in a high-confidence ASD risk gene ortholog, which has not been extensively studied in mice and worms. We are currently fine mapping this region to identify causal genes and variants. Further epistasis analyses between these causal genes and canonical ASD risk orthologs may uncover novel genetic interactions and expand our understanding of ASD risk gene networks and polygenicity.

665B Regulation of associative learning by biogenic amines and their interactions

Melodi Jabaly, Sagi Levy Faculty of Biology, Technion

A central goal of neuroscience is to elucidate the mechanisms underlying associative memory formation. Biogenic amines modulate associative learning in a complex manner, influenced by multiple factors, including the specific amine pathway, training valence and context, and interactions between biogenic amine pathways. While previous studies have provided valuable insights into individual biogenic amines in learning and memory, their roles have not been systematically examined across different amines, their interactions, and varying training conditions.

Here, we present a multidimensional analysis of biogenic amine function in *C. elegans* associative learning, systematically assessing the effects of disrupting individual and paired biogenic amine pathways across diverse valences and contexts. We conditioned animals by pairing either a strong or weak odor cue with the presence (appetitive learning) or absence (aversive learning) of food, then quantified learning by comparing the chemotaxis towards the conditioned odor between naive and trained animals. Our findings reveal distinct roles for specific biogenic amines in memory formation and define the conditions under which they are critical. Moreover, we uncover context-dependent interactions between biogenic amine pathways, including cases where one pathway's effect on learning is unmasked only in the absence of another. For instance, we identify a condition in which disrupting octopamine-related pathways leads to severe learning deficits, while disrupting serotonin alone has no effect—yet, removing both partially restores learning, revealing an interaction between these neuromodulatory systems in aversive learning.

This study provides the first systems-level dissection of biogenic amine contributions to associative learning, with potential implications for conserved neuromodulatory mechanisms in other organisms.

666B Sensory extracellular matrix function and composition in *C. elegans* ciliated neurons

Katherine C Jacobs[1], Deanna M De Vore[2], Karla M Knobel[3], Jonathon D Walsh[1], Navya Malhotra[1], Ken C. Q. Nguyen[4], David H Hall[4], Maureen M Barr[11]Rutgers University, [2]Passaic County Technical Institute, [3]University of Wisconsin-Madison, [4]Albert Einstein College of Medicine

The ability to sense and respond to our environment underlies all of our basic functions. Many of our senses are mediated by sensory neurons that are coated in extracellular matrix (ECM). Little is known about how this "sensory ECM" impacts a neuron's ability to detect environmental cues. Furthermore, we lack an understanding of the composition of sensory ECM. *C. elegans* male-specific ciliated neurons provide an excellent system in which to study the function and composition of sensory ECM.

A genetic screen for regulators of ciliary sensory channel localization revealed a role for *mec-9*. *mec-9* encodes an ECM protein containing conserved Kunitz and EGF-like domains and is required for mechanosensation by non-ciliated touch receptor neurons. We found that the putative neomorphic allele *mec-9(ok2853)* interferes with ciliated sensory neuron function (male mating) and causes ciliary localization defects of the TRP channel protein PKD-2. *mec-9* null alleles do not cause mating defects or PKD-2 phenotypes.

Ciliated neurons shed extracellular vesicles (EVs), which play roles in proteostasis and cell-cell communication. One population of ciliary EVs is released outside the animal while a second population is released within sensory organs. We explored how *mec-9(ok2853)* impacts ciliary EV release using a combination of transmission electron and super-resolution microscopy. *mec-9(ok2853)* mutants display accumulations of EVs in the cephalic and amphid sensory organs. We observed no significant change in the number of environmentally released EVs from the neurons of the male tail.

To determine the cell type supplying MEC-9 to the ECM surrounding ciliated sensory neurons, we examined a transgenic *mec-9*::GFP transcriptional reporter. We found that MEC-9 is not produced by ciliated sensory neurons themselves, but instead supplied cell-non-autonomously by companion cells which may be neurons or glia. Taken together, our results support a model in which changes to the ECM interfere with sensation by causing mislocalization of sensory channels and EV accumulation.

Current work is focused on identifying sensory ECM components using available fluorescent ECM reporters and candidates from the transcriptomes of sensory neurons and glia. Our work indicates that the sensory ECM has a unique composition compared to other tissues and neuron types, suggesting that sensory organs have specialized ECM "recipes" to support their unique functions.

667B Impacts of curcumin and chrysanthemin on imidacloprid induced xenobiotic stress and disrupted cholinergic signaling in a *C. elegans* model

Thea J.A. Jacobsen, Katya Boyce Tjahaja, Scott Boyce Dobrin, Denise B Flaherty Biology, Eckerd College

Imidacloprid is a widely-used pesticide representing over a third of neonicotinoid use in the United States, as of 2015. While pesticides target "pest" species, they can also challenge the health of off-target species like pollinators, decomposers, and humans, affecting ecosystem sustainability and public health. Imidacloprid, a synthetically-derived nicotinic compound which mimics acetylcholine, is intended to target pest-specific nicotinic acetylcholine receptors. This project seeks to determine the impact of imidacloprid on the off-target species, *C. elegans*, by examining the changes induced in mortality and fluorescent GFP-tagged transgenic expression of cholinergic signaling genes (*acr-2* and *unc-17*), as well as, xenobiotic stress genes (*daf-16* and *hsp-16.2*). Furthermore, we sought to determine if the natural antioxidants curcumin and chrysanthemin could ameliorate the detected stress responses. The data indicate that imidacloprid does negatively impact expression of cholinergic signaling in the off-target species, *C. elegans*. Furthermore, the data also suggest that curcumin acts as an antioxidant turned prooxidant based on concentration, a currently debated subject in the literature for flavonoids. Concomitant pesticide-antioxidant exposure indicates varied levels of dose-dependent responses, particularly in stress response pathways, where increased antioxidant concentration suggests heightened gene expression and antioxidant activity at specific levels of imidacloprid. Of additional concern are the non-target pollinator populations at risk of further decline with current imidacloprid usage. Understanding the relationship between the naturally-occurring antioxidants in our food and synthetically-derived pesticides is essential to the creation of just, equitable, and safe-for-consumption pesticide policy. Further research is needed to fully understand how imidacloprid impacts oxidative stress and changes in cholinergic signaling in off-target species

668B EFN-4 and VAB-8 act downstream of MAB-5/Hox to promote QL cell migration

Vedant Jain, Erik Lundquist University of Kansas

Q neuroblasts are a pair of bilateral neuroblasts that are born in the posterior-lateral region of the animal, with QL on the left and QR on the right. Q cells are the anterior sisters of the V5 seam cells. Initially, QR protrudes and migrates anteriorly over V4 seam cell, whereas QL migrates posteriorly over V5 seam cell. The second phase of migration is Wnt dependent and begins after the first phase and the first Q cell division. QL descendants QL.a/p encounter EGL-20/Wnt which is a posteriorly expressed Wnt ligand. This ligand leads to the initiation of canonical Wnt pathway and expression of the MAB-5/Hox transcription factor in QL. *mab-5*expression in QL.a enables migration posteriorly over QL.p, after which QL.a undergoes cell division to generate two daughter cells QL.aa and QL.ap. QL.aa undergoes apoptosis, and QL.ap continues migration posteriorly and differentiates into the PQR neuron. *mab-5/Hox* is both necessary and sufficient for posterior migration, as ectopic expression in QR results in posterior migration of QR.ap (AQR neuron). The genes regulated by MAB-5 to drive posterior migration have remained unknown. Q cells from *wild-type*, *mab-5* loss-of-function (lof), and *mab-5* gain-of function (gof) strains were FACS sorted and subject to RNA-seq. Differential expression analysis identified genes with increased or decreased expression in these mutants. Expression of the unconventional kinesin gene *vab-8* was reduced in *mab-5* lof and increased in *mab-5* gof. In *vab-8* mutants, QL.a failed to migrate posteriorly from its birthplace, resulting in PQR at the place of QL division. This suggests that *vab-8* is required for posterior QL.a migration. *efn-4/Ephrin* expression was also reduced in *mab-5* lof. In *efn-4* mutants, QL.a undergoes its initial posterior migration, but after division, QL.ap (PQR) fails to complete the final phase of migration, resulting in PQR residing just anterior to the anus. This suggests that *efn-4* affects a later step of QL.ap migration, and that distinct genes might be regulated by *mab-5* to control each step. Transgenic expression of *vab-8* and *efn*-4 in the Q cells rescued PQR migration, suggesting that *vab-8* and *efn*-4 act autonomously in the Q cells downstream of MAB-5. Using live imaging of mutants, we have been able to decipher the functional roles of these genes in the posterior migration process. In sum, we have identified two genes, *vab-8* and *efn-4*, that act downstream of *mab-5/Hox* in a transcriptional program to control posterior migration.

669B Neurodegeneration and Behavioral Impacts of Uncoupling Mitotoxicant Induced ATP Depletion and Membrane Potential Loss in *C. elegans*

Laura E Jameson[1], Joel N Meyer[21]Nicholas School of the Environment, Duke University, [2]Duke University

Mitochondrial dysfunction is an important aspect of many neurodegenerative diseases. Because neurons are highly energetic and oxidant-sensitive cells and loss of neurons leads to potentially severe pathological impacts, it is important to understand what mitochondrial dysfunction contributes to neuronal morphology changes and apoptosis. Mitochondria are complex organelles responsible for the generation of the majority of the energy used by the cell in the form of ATP, conducted by the passing of electrons along the electron transport chain and the generation of a proton gradient. However, mitochondrial toxicants impact this process in a number of different ways, and prior research indicates that not all forms of mitochondrial dysfunction specifically cause neurodegeneration. In order to assess specificity to neurodegeneration, doses of the uncoupling mitotoxicant carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) were selected as the doses that were calculated to cause mild growth delay of 1 and 10% total body length following developmental exposure. Potential neuron type susceptibility was assessed using fluorescent strains tagging dopaminergic, GABAergic, cholinergic, glutamatergic, and serotonergic neurons, which were assessed for morphological damage in dendrites. All neurons showed degeneration following FCCP exposure except for serotonergic neurons ($p < 0.05$, chi-square analysis). Acute exposure to FCCP showed a dose dependent decrease in whole body ATP (strain PE255) but no change in ratio of oxidized:reduced glutathione (strain JV2) ($p < 0.01$, one way ANOVA with Turkey's post hoc). Further experiments to understand the connection between membrane potential loss via uncoupling in behavior combined with non-chemical dissipation of the proton gradient will allow insight into the relationship of mitochondrial toxicity on neuronal function and degradation. It has been shown that generation of reactive oxygen species (ROS) in mitochondria can impact behavior, including the dopaminergic CEP neuron controlled basal slowing response, and that this behavior is dependent on ROS rather than morphological state (Morton, 2025). It is therefore important to understand not only which mechanisms of mitochondrial dysfunction contribute to neurodegeneration, but also how uncoupling contributes to signaling. Deeper understanding of mitochondrial uncoupling may provide opportunities for preventative treatments.

670B Sensation modulates the temperature-dependence of locomotion and egg-laying in *C. elegans*

Hongfei Ji, Samuel Lasse, Miriam B Goodman Molecular and Cellular Physiology, Stanford University

Ongoing challenges due to climate change, such as population collapse, failure of large-scale animal migrations, and shifts in the seasonal timing of biological events, are largely driven by the variation in environmental temperatures. As climate change continues to disrupt ecosystems, understanding how animals sense and adapt to temperature fluctuations is essential. This study investigates the neural and molecular mechanisms underlying thermosensory regulation of behavior in the model organism *Caenorhabditis elegans*. Preliminary data reveal that the TAX-4 cyclic-nucleotide gated (CNG) channel, a key thermosensory gene, modulates the optimal temperature for egg-laying behavior from 25°C (N2 wild type) to 22°C (*tax-4* mutant), suggesting a direct link between thermosensation and behavioral adaptation. Using high-throughput, automated imaging techniques, we characterize the thermal responses of two behaviors – locomotion and egg-laying – across a broad temperature range (15°C to 35°C with 2C increments). With this method, we are measuring each individual's motility level, proportion of time for quiescence, and egg-laying patterns as a function of temperature. We will employ a multidisciplinary approach to dissect the neural and molecular pathways involved in thermosensory regulation. Using cell ablation we will map the functional circuitry of thermosensory neurons to determine how temperature cues are processed to influence locomotor and reproduction behaviors. Additionally, we will screen and investigate genes linked to TAX-4 activity to identify the molecules responsible for the variation in thermal optima. This study aims to uncover fundamental principles of thermosensory regulation that allows animals to execute adaptive behavioral responses to thermal variations and ultimately the changing climates.

671B How Do Size and Orientation Impact Electricity Response in *C. elegans*?

Ambreen Joy, Antonia Bellapianta, Thea Carr, Senuki Elvitigala, Anusha Mutnuru, Lauren Scott, Victoria Lam, Annalise Totten, Gabrielle Martin, Shane Calle, Sarah Gontowski, Sia Jeswani, Sahasra Karri, Zoe Midthun, Charlotte Roth, Aaron Taylor, Jared YoungBiology, Northeastern University

We are characterizing the mechanisms underlying a running response to alternating current in the nematode *Caenorhabditis elegans*. In *C. elegans*, previous research has shown that alternating current applied to the agar substrate triggers an increase in running speed and motivates the worms to leave food lawns (Tee et. al, 2023).

We show that there is a positive correlation between size and running response to alternating current, using mutant strains of various sizes. Furthermore, we are studying how the size of *C. elegans* in different mutant strains correlates with their responses to varying voltages. We are also studying the relationship between worm orientation and their response to alternating current. Through these experiments, we are exploring more deeply how the worms are affected by the electricity that we apply to the agar substrate.

The assays entail picking 6 worms onto a plate using an eyelash pick with 6 separate lawns of *E. coli*. From there, a circuit apparatus is set up on the agar plate, allowing the current to travel to the worms. Data is recorded thirty seconds prior to voltage application, one minute during voltage application, and thirty seconds after voltage application. The voltage conditions being tested are 30 V at 80 mA, 45 V at 120 mA, and 60 V at 160 mA. The *C. elegans* strains used in our study include the *sma-1* alleles (*sma-1 (e30) and sma-1 (ru18))*, *exc-2, dbl-1*, and the wild-type N2 line. Orientation assays specifically focus on a worm's orientation at electricity onset, and at onset of running, with the specific assay setups varying between teams in the zoom of the camera used and the frame that is recorded. Data analysis of each individual worm, post-assay, is conducted using the TrackMate program on Fiji.

From the data collected, we hope to provide new insight into the field of sensory response and neuroplasticity using this nematode. We expect that worms experience the electricity based on the distance between the head and tail with respect to the electric field, which would be affected predictably by worm size and orientation with respect to the electric field, and should be reflected in their behavior. Future work will be able to leverage the understanding gained to design new and more informed assays that will empower greater understanding of this aversive response to electricity in *C. elegans.*

672B Characterization of cilia regrowth in the adult animal

Kirsten Judge[1], Alison Philbrook[2], Laura Grunenkovaite[1], Piali Sengupta[11]Brandeis University, [2]Rhode Island College

Primary cilia are critical for sensing and transducing external cues. Cilia in dividing cells disassemble upon entry into the cell cycle and regrow upon exit. However, loss of cilia in postmitotic neurons can severely affect cellular functions. For instance, infection of the olfactory epithelium by SARS-CoV-2 is associated with rapid deciliation of olfactory neurons and onset of anosmia. Although olfactory cilia partly regenerate post-infection to restore sensory functions, the mechanisms required for this regrowth are unknown. In *Chlamydomonas*, severed flagella regrow following truncation, and this regrowth is associated with upregulation of intraflagellar transport (IFT) genes required to build cilia. The molecular mechanisms that sense cilia truncation and trigger regrowth in differentiated cells are unclear.

Cilia are present only on sensory neurons in *C. elegans* and are essential for their functions. The bulk of ciliogenesis occurs during embryonic development. We have used an engineered temperature-sensitive allele of the *osm-3* IFT kinesin gene to conditionally truncate and regrow cilia in adults. We find that a subset of genes previously implicated in axon regeneration partly contributes to cilia regrowth. Preliminarily, we find that levels of IFT proteins are transiently regulated during cilia regrowth but exhibit gene-specific patterns of temporal regulation. Embryonic ciliogenesis is regulated by the DAF-19 RFX transcription factor which drives the expression of all IFT genes. To determine whether DAF-19 functions are also required in the adult, we engineered a *daf-19::AID* allele to conditionally degrade DAF-19 in the presence of auxin. We find that conditional degradation of DAF-19 in a cell-specific manner has no effect on cilia in adult wild-type animals suggesting that continuous DAF-19 function is not necessary to maintain cilia in the adult. However, depletion of DAF-19 specifically during truncation or regrowth in the *osm-3(ts)* background inhibits regrowth, indicating that DAF-19 function is necessary for regrowth in the adult. We are additionally characterizing molecules identified via forward genetic screens that affect cilia regrowth in the adult without affecting developmental ciliogenesis. Identification of the mechanisms regulating cilia regrowth in adult neurons may describe pathways regulating ciliary structural homeostasis and allow for the restoration of cilia structure and function following injury or disease.

673B Exploring cell cycle regulation of sex-specific neuronal development

Andrea Kalis, Joseph Park, Jennifer Ross Wolff Carleton College

Sexually distinct programs of survival, division, and differentiation are key to development of neural circuits driving reproductive behavior. In *C. elegans*, several neuroblasts of the P lineage divide in males, but not in hermaphrodites, initiating divergent programs of differentiation in the ventral cord and tail. Most male-specific neurons are generated during L3, after the majority of sex-shared neurons have ceased dividing. Among these "late bloomers" are the Pn.aap neuroblasts, which generate CA and CP neurons that influence mating movements and sperm transfer. Pn.aap neuroblasts divide in males to generate nine CA/CP neuron pairs, and either die or differentiate in hermaphrodites to produce six VC neurons, which modulate egg-laying. These sex-specific programs depend on the sex determination factor TRA-1 and the Hox proteins MAB-5 and LIN-39.

While it is clear that the sex determination pathway defines Pn.aap sexual dimorphism, the regulators of cell division and differentiation timing remain unknown. We are interested in identifying these regulators and addressing whether cell division is necessary and/or sufficient to direct Pn.aap neuroblasts to assume male-specific identities. The cell-cycle sensor mcm-4p::cdksensor::gfp (CCS) allows us to observe the Pn.aap neuroblast divisions in real time: CCS is expressed male-specifically in the ventral cord beginning in late L2, with GFP expression becoming cytoplasmic in early-mid L3, indicating cell cycle commitment. Male Pn.aap cells divide in mid-late L3 and continue to express GFP in both daughters through L4. The Pn.aap neuroblasts in Pmasc, an XX strain in which the P lineage has been masculinized, adopt the male-typical expression pattern of CCS.

To identify regulators of the Pn.aap cell cycle, we have taken a candidate approach and focused on regulators that influence cell cycle progression, including the CIP/KIP family of CDK inhibitors (*cki-1*, *cki-2*). Double RNAi knockdown of *cki-1* and *cki-2* results in supernumerary division of the Pn.aap neuroblast in hermaphrodites suggesting that the CKIs inhibit Pn.aap cell division. Effects of the double knockdown in males include abnormal CP neuron differentiation. Further study of Pn.aap division and differentiation in males, hermaphrodites, and Pmasc will shed light on the role of Hox proteins and other key regulators in both cell-autonomous and non-autonomous aspects of sex-specific neuronal development.

674B The tardigrade as an emerging model organism for systems neuroscience

Ana M Lyons, Saul Kato Neurology, UCSF

We present the case for developing the tardigrade (*Hypsibius exemplaris*) into a model organism for systems neuroscience, leveraging knowledge and tools from the model organisms *C. elegans* and *D. melanogaster*. These microscopic, transparent animals (~300-500 microns) are among the smallest known to possess both limbs (eight) and eyes (two), with a nervous system of only a few hundred neurons organized into a multi-lobed brain, ventral nerve cord, and a series of ganglia along the body. Despite their neuroanatomical simplicity, tardigrades exhibit complex behaviors, including multi-limbed walking gaits, individual limb grasping, phototaxis, and transitions between active and dormant states. These behaviors position tardigrades as a uniquely powerful system for addressing certain fundamental questions in systems neuroscience, such as: How do nervous systems coordinate multi-limbed behaviors? How are top-down and bottom-up motor control systems integrated? How is stereovision-guided navigation implemented? What mechanisms underlie neural resilience and recovery during environmental stress? We review current knowledge of tardigrade neuroanatomy, behavior, and genomics, and we identify opportunities and challenges for leveraging their unique biology. We propose developing essential neuroscientific tools for tardigrades, including genetic engineering and live neuroimaging, alongside behavioral assays linking neural activity to outputs. Leveraging their physiological and evolutionary relationships to *C. elegans* and *D. melanogaster*, we can adapt existing toolkits to accelerate tardigrade research - providing a bridge between simpler invertebrate systems and more complex neural architectures.

675B Characterizing dynamic spatiotemporal requirements of neurodevelopmental disorder risk gene orthologs using Auxin-inducible degradation in *Caenorhabditis elegans*

Lexis D.M. Kepler[1], Troy A McDairmid[2], Isabel S Wilson[3], Catharine H Rankin[3,41]1.Djavad Mowafaghian Centre for Brain Health, University of British Columbia, [2]Genome Sciences University of Washington School of Medicine, [3]Psychology, University of British Columbia, [4]Djavad Mowafaghian Centre for Brain Health, University of British Columbia

Neurodevelopmental disorders (NDDs) are characterized by a range of behavioural, communication, and motor impairments which can significantly impact an individual's quality of life. While hundreds of NDD-risk genes have been recently identified, there is an overwhelming need to characterize how and when gene disruptions contribute to disorder phenotypes. While the spatiotemporal requirements can be predicted based on expression data, conditional knock-in/out rodent studies suggest that some NDD-risk gene orthologs carry out important functions well beyond the peak expression period, and gene re-expression in adulthood can improve alterations. However, due to the high costs and technical demands of rodent studies, the spatiotemporal requirements of gene function for NDD-associated behaviours and motor traits have only been validated for a handful of risk genes. Here, we used our high-throughput strategy to assess the spatiotemporal requirements of NDD risk gene orthologs using a conditional protein degradation system and machine vision phenotypic profiling in *Caenorhabditis elegans*. Using this approach, we measured the effects of degrading and re-expressing 10 NDD-risk gene orthologs across 30 morphological, locomotor, sensory, and learning phenotypes at multiple time points throughout development. We found that the spatiotemporal requirements of gene function and degree of phenotypic reversibility heavily varied by gene and phenotype. For example, a subset of genes displayed were required throughout development, while others showed functional windows restricted to early development. Additionally, the re-expression of some genes improved multiple phenotypic alterations across broad developmental time windows, while others only improved a subset of the phenotypes. This high-throughput approach offers insights into neural development and the spatiotemporal requirements of gene function for multiple behaviours implicated in NDDs.

676B Tools for analysis of next-generation whole-brain imaging modalities

Rex Kerr[1], Muneki Ikeda[1], Saul Kato[21]University of California at San Francisco, [2]Neurology, University of California at San Francisco

Live imaging in model organisms has benefitted from rapid advances in camera technology and microscopy techniques, although new approaches often introduce novel technical challenges. For example, single-objective light-sheet microscopy, such as SCAPE, can perform calcium imaging on freely-moving whole adult worms at 10+ volumes per second with one micron or better resolution along every axis. However, both the nature and the quantity of the data can challenge existing analysis pipelines.

A single high-speed high-sensitivity camera can produce data at rates of 4+ GB/s, generating terabytes of data for an experiment that lasts a few minutes, posing problems both for long-term storage and data browsing. We address this by computing projections and downsampled copies for fast browsing; losslessly compressing background-subtracted data to reduce storage space by 100x; and making use of parallel processing.

Another challenge arises from SCAPE traversing the sample at an oblique angle, giving a non-orthogonal volumetric data layout. Resampling to an orthogonal space eases analysis but can blur signals. Thus, we favor an approach that produces regions of interest on the resampled data that can then be inversely transformed to measure signal in the raw data, with subvoxel accuracy.

Neuron segmentation and identification is closest to being a solved problem for spinning-disk confocal microscopy. To take advantage of this, we capture multi-color confocal stacks for each animal used for SCAPE live imaging. We then register SCAPE head-subvolumes with each other and with the confocal reference, enabling segmentations and identities found in the confocal data to be applied to the raw SCAPE data. We also generate data mapped to head-centric coordinate space in Neurodata Without Borders format for wider compatibility.

Our tools will be available to the worm community on WormID.org to collectively solve the technical challenges associated with advanced microscopy techniques.

677B The kpc-1 3'UTR facilitates dendritic transport and translation efficiency of mRNAs for dendrite arborization of a mechanosensory neuron important for male courtship

Eunseo Kim Biological Science, University of Illinois at Chicago

A recently reported Schizophrenia-associated genetic variant in the *3' UTR* of the human furin gene, a homolog of *C. elegans kpc-1*, highlights an important role of the furin *3' UTR* in neuronal development. We isolate three *kpc-1* mutants that display abnormal dendrite arborization in PVD neurons and defective male mating behaviors. We show that the *kpc-1 3' UTR* participates in dendrite branching and self-avoidance. The *kpc-1 3' UTR* facilitates mRNA localization to branching points and contact points between sibling dendrites and promotes translation efficiency. A predicted secondary structural motif in the *kpc-1 3' UTR* is required for dendrite self-avoidance. Animals with over-expression of DMA-1, a PVD dendrite receptor, exhibit similar dendrite branching and self-avoidance defects that are suppressed with *kpc-1* over-expression. Our results support a model in which KPC-1 proteins are synthesized at branching points and contact points to locally down-regulate DMA-1 receptors to promote dendrite branching and self-avoidance of a mechanosensory neuron important for male courtship.

678B Argonaute-mediated small RNA pathways mediate maternal age-dependent behavioral plasticity in *C. elegans*

Taehyun A Kim[1], Hyeonjeong A Hwang[1], Rinho Kim[2], Kyuhyung Kim[11]Brain Sciences, Daegu Gyeongbuk Institute of Science and Technology, [2]Department of Totipotency, Max Planck Institute of Biochemistry

Parental age influences progeny fitness, yet the molecular mechanisms underlying these effects remain largely unknown. Previously, our lab has shown that *C. elegans* progeny from old mothers exhibit reduced avoidance behavior toward a pheromone ascaroside#3 (ascr#3). This maternal-age-dependent behavioral change is associated with decreased expression of the *eri-1* exoribonuclease gene in the AVH interneurons. These findings suggest that a small RNA pathway modulates maternal-age-dependent neural activity, ultimately modulating behavioral outcome. However, the molecular and neuronal mechanisms in which small RNA pathways coordinate these processes across generations remain to be uncovered. To investigate the roles of small RNA pathways underlying maternal age-dependent behavioral plasticity, we first sought to identify which Argonaute proteins, the core components of RNA-induced silencing complexes (RISC), mediate ascr#3 avoidance behavior. We tested 19 out of 27 Argonautes in *C. elegans*, and found that the mutants of *alg-3, alg-5, ergo-1,* and *wago-10* exhibited significantly reduced ascr#3 avoidance behavior. We then examined the expression patterns of these genes and found that *alg-3* was expressed in the pheromone-sensing ASI neurons, while *ergo-1* was predominantly expressed in the germline but also in unidentified head neurons or glia. We are currently performing rescue experiments to determine whether the expression of Argonaute genes restores ascr#3 avoidance defects. While *eri-1* expression in the AVH interneurons is influenced by maternal age, its role in AVH function and the associated regulatory networks remain unclear. To investigate this, we are currently performing TRAP-sequencing to identify upstream and downstream factors. This research will elucidate how small RNA pathways coordinate gene regulatory networks to mediate behavioral plasticity across generations.

679B modWorm: Modular simulation framework for modeling and integrating neural connectomics, dynamics and biomechanics for *Caenorhabditis elegans*

Jimin Kim[1], Jeremy Florman[2], Linh Truong[1], Julia Santos[3], Mark Alkema[2], Eli Shlizerman[11]Electrical and Computer Engineering, University of Washington, [2]University of Massachusetts Medical School, [3]University of Washington

Computational approaches which emulate *in-vivo* nervous system are needed to investigate mechanisms of the brain to orchestrate behavior. Such approaches must integrate a series of biophysical models encompassing the nervous system, muscles, biomechanics to allow observing the system in its entirety while supporting incorporations of different model variations. Here we develop *modWorm*: a modeling framework for the nematode *Caenorhabditis elegans* using *modular integration* approach. modWorm allows for construction of a model as an integrated series of configurable, exchangeable *modules* each describing specific biophysical processes across different modalities (e.g., nervous system, muscles, body). Utilizing modWorm, we propose a base neuro-mechanical model for *C. elegans* built upon the complete *connectome.* The model integrates a series of 7 modules: i) intra-cellular dynamics, ii) electrical and iii) chemical extra-cellular neural dynamics, iv) translation of neural activity to muscle calcium dynamics, v) muscle calcium dynamics to muscle forces, vi) muscle forces to body postures and vii) proprioceptive feedback. We validate the base model by *in-silico* injection of constant currents into sensory and inter-neurons known to be associated with locomotion behaviors and by applying external forces to the body. Applications of *in-silico* neural stimuli experimentally known to modulate locomotion show that the model can recapitulate natural behavioral responses such as forward and backward locomotion as well as mid-locomotion stimuli induced responses such as avoidance and turns. Furthermore, through *in-silico* ablation surveys, the model can infer novel neural circuits involved in sensorimotor behaviors. To further dissect mechanisms of locomotion, we utilize modWorm to introduce empirical based variations of intra and extra-cellular dynamics as well as model optimizations on associated parameters to elucidate their effects on simulated locomotion dynamics compared to experimental findings. Our results show that the proposed framework can be utilized to identify neural circuits which control, mediate and generate natural behavior.

680B Spatiotemporal Traction Force Measurement Toward Understanding the Locomotion Mechanism of *C. elegans*

Ryota Kimura[1], Takashi Morikura[1], Shogo Miyata[2], Yuki Tsukada[3], Akira Funahashi[31]Center for Biosciences and Informatics, Keio University, [2]Department of Mechanical Engineering, Keio University, [3]Department of Biosciences and Informatics, Keio University

The nematode *C. elegans* exhibits undulatory locomotion on an agarose gel surface. Like many animals, the locomotion of *C. elegans* depends on the thrust force generated through interactions between its body and the surrounding environment. Although many previous studies have focused on the neural and genetic aspects of *C. elegans* locomotion, the detailed physical mechanisms remain unclear. In this study, we investigated the spatiotemporal mechanics of *C. elegans* locomotion on the agarose gel by measuring the forces generated in the environment. To achieve this, we employed traction force microscopy, which reconstructs forces based on the observation of the displacements of markers embedded in the substrate.

First, we placed the TP12 strain of *C. elegans*, which expresses GFP-tagged collagen COL-19 on its epidermis, onto an agarose gel embedded with fluorescent beads as markers. We then captured time-series images of locomotion using confocal microscopy. For force reconstruction, it is essential to use the precise mechanical properties of the agarose gel prepared with S-buffer as the solvent, as employed in this study. We obtained these properties by performing both compression and relaxation tests using a displacement-controlled uniaxial compression apparatus. Based on these measurements, we estimated the in-plane forces generated at the contact region between the nematode's body and the substrate.

Our mechanical property measurements indicated that the agarose gel exhibits viscoelastic behavior largely dominated by elasticity, with a Young's modulus on the order of tens to hundreds of kPa. From the estimated spatiotemporal force field, we qualitatively observed pronounced normal force components directed toward the centerline of the nematode's body, which we infer originate from adhesion caused by the meniscus formed by the liquid film on the gel. Meanwhile, since we also observed force components likely related to thrust, we are currently conducting a quantitative analysis and developing a model that correlates the measured forces with the nematode's displacement.

This study not only deepens our fundamental understanding of the mechanics underlying microscale locomotion on lubricated wet surfaces but also provides an experimental basis for further exploration of the neural mechanisms controlling behavior.

681B GABA and acetylcholine signaling modulate motor response plasticity in *C. elegans*.

Justin O King, Samantha M Kobal, Merve Arslan, Jacqueline K Rose Psychology, Western Washington University

The role of GABA and acetylcholine in mobility has been well described. We explored how these signaling pathways influence learning in *Caenorhabditis Elegans (C. elegans)*. Previous findings from our lab and others have shown that the cholinergic agonist nicotine, and the GABA agonist toluene, both affect mobility. Our lab has also found that toluene's effects on mobility correspond to increased SNB-1 (synaptobrevin) expression in GABA-releasing neurons. This result was supported by qPCR results showing increased *unc-47* transcripts following toluene exposure. To test if GABA and acetylcholine signaling pathways influence learning, we employed an associative conditioning protocol previously described by our lab. Briefly, two stimuli (a mechanical vibration and a blue light stimulus) elicit activity in two opposing motor response circuits are repeatedly delivered together. The result of these stimulus pairings is an altered motor response at test during which worms are exposed to a single stimulus. To activate cholinergic signaling at the time of conditioning, we trained *punc-17*::ChR2 worms such that the blue light introduced during vibration-blue light conditioning activated both the endogenous blue light response and the channelrhodopsin expressed in cholinergic neurons. In a similar set of trials, we conditioned *punc-47*::ChR2 worms so that GABAergic neurons were activated at the time of associative conditioning with vibration and blue light. Results indicate the learned behavioral response was altered when either of these signaling pathways were activated during conditioning. Our findings from toluene and nicotine exposure—as well as from oppositional learning using optogenetics—position us for future research on conditions that affect learning and could eventually elucidate the effects of toluene and nicotine consumption and abuse on learning.

682B Investigating the functions of F-actin regulators in neurodevelopment in *C. elegans*

Sydney Ko[1], Kota Mizumoto[21]Cell and Developmental Biology, The University of British Columbia, [2]Zoology, The University of British Columbia

The formation of functional neural circuits depends on several neurodevelopmental processes, including neurite outgrowth, axon branching, and synapse formation. These processes require branched filamentous actin (F-actin) formation by the Arp2/3 complex and its regulators, such as the Arp2/3-activating WAVE complex and Arp2/3-inhibiting Coronins. Despite this, the precise roles of branched F-actin regulators in neurodevelopment remain elusive as their loss of function mutants are embryonic lethal. To directly test the functions of branched F-actin regulators in neurodevelopment, we use the auxin-inducible degron (AID) system to knock down branched F-actin regulators, specifically in the nervous system (Kurashina & Mizumoto, 2023; Zhang et al., 2015). Using the AID system, we found that ARX-2/Arp2, a subunit of the Arp2/3 complex, WVE-1/WAVE, and POD-1/CORO7 are necessary for synapse formation but not for maintenance in the DA9 motor neuron. Specifically, continuous neuronal knockdown of ARX-2/Arp2 and WVE-1/WAVE throughout larval development resulted in decreased, whereas continuous POD-1/CORO7 knockdown resulted in increased synapse numbers in DA9 at the L4 stage compared to wild type. On the other hand, synapse numbers were unaffected in day 1 adult animals when we induced knockdowns at the L4 stage. These results confirm previous work showing that branched F-actin is required only during synaptogenesis but not for the maintenance of existing synapses (Chia et al., 2014). Furthermore, we found that ARX-2/Arp2 is necessary for axon branch formation in the PLM mechanosensory neurons. Specifically, continuous neuronal knockdown of ARX-2/Arp2 throughout larval development resulted in the loss of axon branches of the PLM neurons. Currently, we are investigating the functions of WVE-1/WAVE and POD-1/CORO7 in PLM axon branch formation and maintenance and the genetic and molecular mechanisms that mediate the localization of branched F-actin regulators in neurodevelopmental processes.

683B Beyond sight and smell: The multifunctional LITE-1 receptor in *Caenorhabditis elegans*

Alan Koh[1,2], Eduard Bokman[3], Alexey Gavrikov[4], Javier Rodriguez[1,2], Changchun Chen[1,2], Alon Zaslaver[3], André Brown[1,21]MRC Laboratory of Medical Sciences, London, [2]Institute of Clinical Sciences, Imperial College London, [3]Department of Genetics, Silberman Institute of Life Science, The Hebrew University of Jerusalem, [4]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences

The ability to sense and respond to environmental stimuli is fundamental across the biological spectrum, allowing organisms from bacteria to complex eukaryotes to respond to their environment. The nematode Caenorhabditis elegans has evolved a diverse array of sensory receptors to perceive various environmental stimuli. One such receptor, the gustatory homolog LITE-1, was initially identified for its essential role in mediating *C. elegans* aversive responses to blue and ultraviolet light.

Our recent findings reveal that LITE-1 also functions as a chemoreceptor, expanding its role beyond photoreception and bridging two distinct sensory modalities, light and smell. This dual function highlights LITE-1 versatility in perceiving and integrating two different types of stimuli. Our data are consistence with LITE-1 functioning as an ion channel that is capable of directly detecting volatile organic compounds commonly associated with prey detection. Molecular docking simulations indicate that these ligands fit within LITE-1 putative chromophore binding pocket. Suggesting the possibility that LITE-1 photosensory may be activated by a chromophore or a bacterial secondary metabolite. This raises intriguing questions regarding the potential interplay between its light sensing and chemosensory functions.

Ongoing investigations into the neural circuitry underlying LITE-1 sensory versatility suggest that its chemosensory pathway is distinct from its cGMP dependent phototransduction cascade. This distinction underscores the complexity of LITE-1 mediated signalling and its broader implications for sensory integration in *C. elegans*. Understanding the mechanisms that enable LITE-1 to function as both a photoreceptor and chemoreceptor will provide insights into how sensory systems evolve to detect and integrate diverse environmental signals.

684B Behavioral and genetic evidence that habituation involves dissociable, interstimulus interval-sensitive processes

Nikolas Kokan[1], Annika Bodlt[2], Yvette Ni[2], Shawn Yee[2], Abhyudai Singh[3], Catharine Rankin[21]Neuroscience, University of British Columbia, [2]University of British Columbia, [3]University of Delaware

Habituation is a form of learning that occurs when an organism decreases its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. The time between stimulus presentations greatly impacts the rate, depth, and memory of habituation. The goals of this research are 1) to provide a deeper understanding of how the time between stimulus presentations - the interstimulus interval (ISI) - influences habituation, and 2) to identify genes that have an ISI-dependent effect on habituation. Animals habituate slowly and to a lesser extent at long ISIs, but the response decrement persists for longer than when the decrement is induced by short ISIs. Thus, at longer ISIs habituation learning is slower, but memory of this learning lasts longer than at shorter ISIs. To investigate whether this is caused by different habituation processes acting at short and long ISIs, we used our Multi-Worm Tracker to simultaneously monitor the behavior of dozens of*Caenorhabditis elegans*, on Petri plates that receive mechanical taps at different ISIs. The worm's naive response to a tap is to reverse, moving backwards briefly. While we found that this reversal response habituated more deeply with short ISIs (10-30s), once the ISI reached 60s habituation did not get any shallower as the interval was increased to 300s. Memory of habituation after 5 minutes was better at longer ISIs, in contrast when the ISI was altered mid-experiment from 10s to 60s, the response mostly recovered within a single 60s interval, demonstrating the transience of habituation at shorter ISIs. We found that we could mathematically model this habituation data with a transient process that is prominent at short ISIs and a more persistent process important for habituation at long ISIs. We also tested worms with mutations in candidate genes at short and long ISIs and found mutants with habituation defects that vary with the ISI. For example, animals with mutations in *cmk-1*, homolog of Ca2+/calmodulin-dependent kinases 1/4, *ogt-1*, homolog of O-GlcNAc transferase, and *acy-1*, homolog of adenylyl cyclase 9, have a habituation phenotype close to wildtype at one ISI, but a clear mutant phenotype at a different ISI. These genes may act in ISI-sensitive habituation processes as their habituation phenotype depends on the ISI. This is predicted by our modeling of habituation and has implications for how the nervous system integrates stimuli separated by different time intervals.

685B Regulation of IDA-1 by the DAF-7/TGF-β pathway in *C. elegans*: A developmental and molecular investigation

Alexandra Kovalenko, Annette McGehee Suffolk University

Environmental factors such as overcrowding, food scarcity, and high temperatures influence both behavioral and molecular responses in *Caenorhabditis elegans*, partly through the DAF-7/TGF-β signaling pathway. This pathway plays a crucial role in regulating developmental plasticity, including the decision to enter the dauer stage under unfavorable conditions. Previous studies have shown that mutations in *daf-7* lead to increased expression of the glutamate receptor GLR-1, suggesting that DAF-7 signaling regulates neuronal gene expression. Our lab is investigating whether this pathway also regulates IDA-1, a protein associated with dense core vesicles (DCVs), which are essential for neuropeptide signaling. We used fluorescence imaging of an IDA-1::GFP reporter strain to determine whether *daf-7* regulates IDA-1 at the protein level. These experiments revealed that *daf-7* mutants exhibit increased IDA-1::GFP fluorescence intensity compared to wild-type worms, supporting the idea that IDA-1 protein levels are regulated by the DAF-7/TGF-β pathway. However, the mechanism of this regulation - whether transcriptional or post-transcriptional - remains unclear. To address this, we analyzed *ida-1* mRNA levels using qPCR in *daf-7* mutants and wild-type worms across key developmental stages (L2, L4, young adult, and adult). By measuring *ida-1* mRNA levels in different strains and at multiple developmental stages, we aim to assess whether its expression is developmentally regulated and whether differences in IDA-1 levels result from transcriptional upregulation or post-transcriptional mechanisms. Understanding how *ida-1* expression changes over time may offer insights into the molecular mechanisms that regulate transcription and influence developmental responses in *C. elegans*, particularly in relation to environmental adaptation and neuropeptide signaling.

686B Mechanisms of FSHR-1 cross-tissue regulation of neuromuscular function in diverse physiological conditions

Lillian Rademacher[1], Milica Nenadovich[1], Alexandra R Alva[1], Ryan R Adkins[1], Lauryn R Padgett[1], Shums Parkar[1], Joshua Gerber[1], Jennifer R Kowalski[21]Butler University, [2]Biological Sciences, Butler University

Maintenance of neuronal signaling balance and nervous system function in response to differing physiological conditions, such as oxidative stress, requires integrated signaling between tissues involved in immunity, metabolism, hormone regulation and the nervous system. Indeed, communication between the intestine, neurons, and glia has been documented in multiple organisms. However, the mechanisms by which this inter-tissue signaling occurs are not well understood. G protein-coupled receptors (GPCRs), because of their broad expression patterns, are unsurprisingly involved in multiple aspects of neuronal signaling and in coordinating multi-tissue responses to stimuli. We recently showed FSHR-1, the sole *Caenorhabditis elegans* ortholog of a family of vertebrate glycoprotein (GP) receptor GPCRs, is necessary and sufficient in the intestine, and can also act in glia and potentially head neurons, to regulate neuromuscular junction (NMJ) signaling balance by promoting synaptic vesicle exocytosis from cholinergic motor neurons. The ability of intestinal FSHR-1 to regulate muscle excitation requires both of its GP ligands, GPLA-1 and GPLB-1, in addition to several downstream effectors (GSA-1, ACY-1, and SPHK-1) in the intestine. FSHR-1 is also implicated in responses to a variety of stresses, including oxidative stress. We hypothesized that FSHR-1 acts downstream of GPLA-1 and GPLB-1 and/or other ligands in the intestine to promote intestinal peptide release that differentially regulates muscle excitation in distinct oxidative stress conditions. Here, we show genes encoding several insulin-like peptides, as well as the secretion regulator, *hid-1*, are required in the intestine for the effects of intestinal *fshr-1* on NMJ function. Further, we found *fshr-1* is required to promote NMJ function under chronic, low-level paraquat-induced oxidative stress but is needed to prevent declines in NMJ function under high-level, acute paraquat stress. The defects in resistance to chronic stress correlate with decreased lifespan of *fshr-1*(*ok778*) loss-of-function mutants. Expression of *fshr-1* in the intestine is sufficient to restore wild type NMJ function in chronic oxidative stress; however, *fshr-1's* role in modulating NMJ activity under these conditions appears independent of at least one of its known GP ligands. Current work is aimed at identifying relevant FSHR-1 ligands, downstream signaling partners, and sites of action required for its cell non-autonomous effects on NMJ regulation under both physiological and oxidative stress conditions. Disruptions in mammalian GP receptor signaling are implicated in depression and affective disorder phenotypes and in neurodegenerative diseases also linked to oxidative stress; thus, our findings may have implications in understanding these human neurological conditions.

687B UNC-43/CaMKII regulates presynaptic assembly in *C. elegans*

Mizuki Kurashina[1,1], Hayley Ding[1], Jolie Wu[2], Teagan Richman-Taylor[2], Kota Mizumoto[11]Zoology, University of British Columbia, [2]Biology, University of British Columbia

Neurons communicate via an interface known as the synapse, comprised of a pre- and postsynaptic specializations. At the presynaptic sites, a group of conserved proteins form an electron dense region called active zone, where active zone proteins regulate the recruitment and release of neurotransmitter-containing synaptic vesicles (SVs). Using the marker strain expressing 3×GFPnovo2::CLA-1 and TdTomato::RAB-3, which label active zones and SVs, respectively, we found that *unc-43,* which encodes the ortholog of calcium/calmodulin-dependent protein kinase II (CaMKII), is integral for the proper presynaptic assembly. In *unc-43(n498n1186)* loss-of-function mutants, we observed increased CLA-1 puncta with reduced fluorescence intensity. These puncta are often localized outside of the presynaptic varicosity, suggesting that *unc-43* is required for the proper clustering of CLA-1 at presynapses. Consistently, the signal intensity of CLA-1 puncta is increased in *unc-43(n498)* gain-of-function mutant. To determine which presynaptic proteins are affected in *unc-43* mutants, we labeled endogenous active zone proteins specifically in DA9 using the split-fluorophore method (Kurashina et al., 2024). We found that CLA-1, SYD-2, and RIMB-1 localization are similarly disrupted in *unc-43(n498n1186)* mutants. On the other hand, UNC-10 localization at the presynaptic varicosity appears to be unaffected while the signal intensity is decreased. These results suggest that *unc-43* plays crucial roles in proper presynaptic assembly by regulating the localization of many active zone proteins at presynapses. Neuron-specific, but not muscle-specific knockdown of UNC-43/CaMKII using the auxin-inducible degron (AID) system suggests that UNC-43/CaMKII functions in the presynaptic neuron for proper active zone assembly. Postembryonic degradation of UNC-43/CaMKII using the AID system resulted in disruption of CLA-1 localization, while postembryonic activation of CaMKII using the photo-activatable CaMKII (paCaMKII) rescued presynaptic localiation of CLA-1, suggesting that UNC-43/CaMKII functions to maintain presynaptic structure. To test functional conservation between human CaMKII and *unc-43*, we replaced the endogenous *unc-43* locus with human CaMKIIA (hCaMKIIA). The resulting humanized *C. elegans* exhibited normal locomotion and presynaptic structure, suggesting that the functions of *unc-43* in presynaptic assembly is conserved in human CaMKIIA. We then introduced a recessive (H477Y) and a dominant (K291P) mutation identified in the CaMKII gene of patients with intellectual disabilities. These disease-causing mutations phenocopied presynaptic defects of *unc-43(n498n1186)* and *unc-43(n498)* mutants. Our work reveals a conserved role of CaMKII in regulating the proper assembly of presynaptic components.

688B Glial GABA receptors control glia-neuron crosstalk in *C. elegans*

Melisa Lamberti, Nika R. Bucan, Mark R. Rozencwaig, Sophia R. Lopez, Laura R. Bianchi Department of Physiology and Biophysics, University of Miami

Gamma-amino butyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain. Normal GABA function requires specialized proteins such as biosynthetic enzymes, transporters, and receptors. Defects in these proteins can lead to imbalance of GABA neurotransmission and, consequently, to disease states such as epilepsy and autism spectrum disorder. Studies have shown that both GABAergic neurons and glial cells synthesize and release GABA to maintain neuronal excitatory-inhibitory balance, plasticity, neuroprotection, and other functions. Both neurons and glia cells express functional ionotropic and metabotropic GABA receptors, however, the role of these GABA receptors in the glia cells is still largely unknown. Our hypothesis is that glial GABA receptors control glia-neuron crosstalk by regulating GABA release from glia. To test this hypothesis, we exploit a two-cell system, in the powerful model organism *C. elegans,* consisting of the Amsh glial cell and the ASH nociceptive neuron. We and others have shown that GABA mediates the functional interaction between Amsh glia and ASH neuron and that this is needed in *C. elegans* for responses to the aversive odorant octanol. Using behavioral assays and calcium imaging, we now found that glia-neuron cross talk is disrupted when the GABAA receptors, *unc-49* and *lgc-36*, are knocked down in glia. These data support the idea that *unc-49* and *lgc-36* might regulate GABA release from glia. Using the GABA sensor iGABASnFR, we are currently directly testing this hypothesis. Finally, we are testing the contribution of other glial GABAA and of GABAB receptors to glia-neuron crosstalk. In summary, our results show that GABAA receptors expressed in the plasma membrane of glial cells might regulate glia-neuron cross talk by controlling the release of GABA itself. These findings hold implications for our understanding of GABA signaling in health and disease.

689B The giant KASH protein ANC-1 establishes mechanical stability in aging *C. elegans* neurons

Matthew Laurence[1], Dacotah Northcroft[2], Nicholas Sidik[2], Daniel Elnatan[2], Kang Shen[3,4], GW Gant Luxton[2], Daniel Starr[21]MCB, University of California Davis, [2]MCB, UC Davis, [3]Department of Biology, Stanford, [4]Howard Hughes Medical Institute

Age-related neurodegeneration affects over 50 million people worldwide, yet current therapies show limited success. Mutations in the giant Klarsicht/ANC-1/SYNE homology (KASH) protein Nesprin-1 giant (Nesprin-1G) of the linker of nucleoskeleton and cytoskeleton (LINC) complex are implicated in amyotrophic lateral sclerosis and ataxia, suggesting disrupted nuclear-cytoskeletal coupling contributes to pathogenesis. Using *C. elegans*, we investigate how the Nesprin-1G ortholog ANC-1 affects neuronal mechanical properties and integrity during aging. Previous work in hypodermis and intestine revealed ANC-1 performs dual functions: beyond nuclear positioning, it acts as a mechanical scaffold at the endoplasmic reticulum (ER). We hypothesized this scaffolding function also operates in neurons and contributes to neuronal integrity. Using genetically encoded multimeric nanoparticles (GEM)-based nanorheology in PVD neurons, we found a bimodal distribution of GEM mobility in wild-type neuronal cell bodies: 43% of GEMs exhibit confined motion, while 57% remain mobile. In *anc-1 null* mutants, the confined population decreases to 12% ($p<0.001$). Interestingly, GEM mobility in primary dendrites showed minimal differences between wild-type and *anc-1 null* mutants, suggesting ANC-1's mechanical influence is largely confined to the cell body where it is predominantly localized. Mutations in UNC-84, which partners with ANC-1 to form the LINC complex, show distinct diffusion patterns, revealing separate roles for these components. Confocal microscopy of GFP-tagged endogenous ANC-1 revealed its dynamic localization, shifting from nuclear envelope association in larval stages to dispersed ER distribution with age. In *anc-1 null* neurons, mitochondrial positioning errors increase 3.2-fold with severe detachment from the nuclear envelope, and nuclear displacement increases 2.7-fold ($p<0.01$). In contrast, *unc-84 null* neurons show milder nuclear displacement (1.6-fold) with significantly less mitochondrial detachment (1.4-fold), highlighting ANC-1›s specific role in maintaining nuclear-mitochondrial associations. These organelle positioning defects correlate strongly ($r=0.78$) with dendrite deterioration. At the organismal level, *anc-1 null* animals display significantly reduced motility (47% decrease in body bends) and shortened lifespan ($p<0.001$). By establishing mechanical instability as a primary driver of neural decline, our research offers insights into neurodegeneration mechanisms and suggests novel therapeutic targets.

690B Neurpeptidergic regulation of nictation, a host- and food-seeking behavior in dauers

Tuan Anh Le, Charline Roy, Sharon De Keyser, Ferre Smeets, Liliane Schoofs, Liesbet Temmerman KU Leuven

Dauer or dauer-like life stages of nematodes utilize nictation as a host- and food-seeking behavior, during which nematodes stand upright on their tails, often waving back and forth. In this manner, human parasites and nematodes detrimental to animals, attach themselves to their respective hosts.

Previous work by us and others suggests that neuropeptides may be crucial regulators of nictation in *Caenorhabditis elegans*. In our current work, a genetic screen was performed using loss-of-function mutants of all known genes encoding FMRFamide-like neuropeptides (FLPs). In addition to nictation as the main readout, we evaluated dauer entry and locomotion (swimming and crawling) for each condition. Based on those combined results, individual FLP genes affect distinct aspects of dauer physiology and behavior. Out of all screened candidates, *flp-5*, *flp-6*, *flp-9*, *flp-13*, *flp-15* and *flp-19* can be prioritized as specific regulators of nictation. Furthermore, while several amongst the 14 putative receptors of these FLPs severely affect dauer entry, FRPR-8 can already be proposed as a modulator of nictation.

Overall, we provide insight into the neurogenetics of nictation, unveiling fundamental biological principles by which neurohormones can control behavior. Our findings put forward concrete targets for unravelling dauer biology and for follow-up in economically relevant nematode species.

691B *erh-1* loss of function rescues pathological tau mediated neurodegeneration

Katherine LeBlanc[1], Randall Eck[2,3], Aleen D Saxton[4], Pamela J McMillan[5], Rebecca L Kow[3,4], Brian C Kraemer[1,2,3,4,5]1Molecular and Cellular Biology, University of Washington, [2]Graduate Program in Neuroscience, University of Washington, [3]Division of Gerontology and Geriatric Medicine, University of Washington, [4]Geriatrics Research Education and Clinical Center, [5]Department of Psychiatry and Behavioral Sciences, University of Washington

Alzheimer's Disease (AD) is a progressive neurodegenerative condition characterized by intracellular tau tangles, extracellular beta amyloid plaques, and profound defects in cognition and memory. AD incidence is projected to rise as the world's population ages, highlighting the need to better understand the factors driving neurodegeneration in AD and other related tauopathies. *Caenorhabditis elegans* is an excellent model to study tauopathies, as human tau expression in neurons leads to behavioral deficits, pathological tau accumulation, neurodegeneration, and lifespan decreases, mirroring human disease. Leveraging the genetics of *C. elegans* has allowed for rigorous interrogation of pathways involved in toxic tau pathology via forward and reverse genetic screens and have led to many translationally relevant discoveries, including forming the basis for several clinical drug trials. ERH-1 is one such protein identified through genetic manipulation of a tau transgenic *C. elegans* model. ERH-1 is a small protein located in nuclear speckles and was not previously known to play a role in neurodegenerative disease; however, more is known about the protein in the context of cancer. It is involved in varied processes within the cell including pyrimidine biosynthesis, cell cycle regulation, transcription inhibition, DNA damage repair, splicing, microRNA clustering, meiosis, erythroid differentiation, and gene silencing and has over 200 known binding partners. Knockout of ERH-1, one of the two *C. elegans* homologues of human ERH, improves behavior, ameliorates neurodegeneration, and decreases total and phosphorylated tau levels in tau transgenic strains. Investigation of human tissue also points to an association between ERH levels and AD in the frontal cortex, indicating that this discovery may have translational relevance. RNA sequencing suggests that ERH-1 may be altering transcripts involved in the unfolded protein response and it is a known binding partner of another group of tau modulators, the ALYREF proteins (PMID: 35122183), indicating several pathways that ERH-1 might act through to modulate tau pathology. By using *C. elegans*, we hope to better understand the molecular and cellular mechanisms of tau toxicity to broaden the understanding of tauopathies and lead to new treatments.

692B A single pair of associatively-coupled inputs induces independent learning across sub-networks in the *C. elegans* nervous system

Eugene L.Q. Lee, H. Robert Horvitz Biology, HHMI, Dept. Biology, MIT

On an organismal level, learning is often quantified as changes in the magnitude of a single acquired or modulated behavior. Yet organisms are capable of expressing a wide range of behaviors mediated by different neural circuits with distinct associated hierarchies and timescales. How learned behaviors are coordinated across different circuits within an individual to execute appropriate actions is an open problem. Here we demonstrate that *C. elegans* can be trained to modulate its feeding behavior through a highly temporally-resolved associative conditioning task in which a neutral odor is paired with an aversive light stimulus in a trial-by-trial paradigm – specifically, we trained worms to inhibit feeding behavior in response to the previously neutral odor. Feeding behavior (pharyngeal pumping) is enacted by the pharyngeal nervous system – a bounded neural circuit distinct from the rest of the nervous system. There is only a single direct synaptic connection through the two somatic RIP neurons that bridges the pharyngeal and the somatic nervous systems, forming a bottleneck architecture of neural sub-networks within the *C. elegans* nervous system. This compartmentalized sub-structure is analogous to how the vagus nerve serves as a bottleneck between the central nervous system and the viscera innervation in other animals. Our finding of pharyngeal nervous system-associated learning behaviors reveals that a viscera-associated nervous system is capable of flexible behavior that extends beyond a purely reflexive nature. We also observed that the same associative pairing task resulted in learned locomotory reversals to the odor post-training. Analyzing individual worm responses revealed that pharyngeal and locomotor learning appear to occur independently of each other, with differential kinetics and magnitudes. These observations indicate that a single pair of associative inputs can produce separate learned behaviors — possibly a consequence of the bottleneck circuit architecture — across distinct nervous system sub-networks within an individual animal. This organization allows for a modularity to how learned behaviors are acquired and expressed, suggesting that there is a repertoire of learned states and actions that an animal can call upon flexibly to enable optimal adaptation to a changing environment.

693B Natural genetic variation in sexually dimorphic exploratory behavior of *C. elegans*

Harksun Lee, Sylvia E.J. Fischer, Dennis H Kim Division of Infectious Diseases, Department of Pediatrics, Boston Children's Hospital and Harvard Medical School

Natural variation in sexually dimorphic behaviors and their molecular underpinnings are poorly understood. In Caenorhabditis elegans adult males exhibit a sexually dimorphic mate-searching behavior involving the increased leaving of a patch of bacterial food compared with hermaphrodite animals that remain on the food. We recently identified from wild strains of C. elegans genetic variants in a specific isoform of the gap-2 gene, encoding a GTPase-activating protein acting on the Ras pathway, which acts in the ADE pair of mechanosensory neurons to exert large effects on a foraging behavior characterized by exploratory roaming and exploitative dwelling states [Lee et al. (2024), Sci. Adv. 10, adk9481]. Here, we show that the expression of gap-2j in the ADE neurons is regulated in a developmental and sexually dimorphic manner. The gap-2j gene was expressed in the ADE neurons during larval development of hermaphrodites and males and in hermaphrodites during adulthood, but was found to be repressed specifically in adult males, causing the markedly increased mate-searching and roaming behavior of adult males. As a consequence, we observed that natural variants that diminish gap-2j activity increased the roaming behavior of hermaphrodite animals without affecting the corresponding behavior of adult males. Our data suggest that common genetic variants of gap-2j prevalent in wild isolates can toggle a sexually dimorphic developmental genetic switch to cause marked changes in sexually dimorphic exploratory behavior of C. elegans.

694B A *C. elegans* Model of Fanconi Anemia Neurological Syndrome

Jessica E Leighton[1], Meagan E Collins[1], Nikita B Noyes-Martel[1], Quintin C Palmer[1], Camila Vila Bolano[1], Belinda Barbagallo[2], Niall G Howlett[11]University of Rhode Island, [2]Salve Regina University

Fanconi anemia (FA) is a human genetic disease characterized by heterogeneous congenital abnormalities and increased risk for bone marrow failure and cancer. Central nervous system defects, including white matter brain lesions and early-onset cognitive decline, have become increasingly recognized in FA patients. This constellation of neurological symptoms is referred to as Fanconi Anemia Neurological Syndrome (FANS). The molecular origins of FANS are unknown.

Multi-omics approaches from our laboratory have uncovered novel connections between the FA proteins and the nervous system. For example, ChIP-seq analysis has revealed that the FANCD2 protein binds to transcriptionally active large neural genes under conditions of replication stress, including genes that function in neuronal differentiation, neurotransmission, and neural cell adhesion. Many of these genes are linked to neuropsychiatric and neurodevelopmental disorders, including schizophrenia, autism spectrum disorder, and intellectual disability.

To study the role of the FA pathway in nervous system development and maintenance, we have turned to the model nematode *Caenorhabditis elegans*; *C. elegans* has a well-characterized nervous system with 302 neurons and nervous system connectivity has been anatomically mapped at high resolution. Quantitative behavioral analysis of deletion strains of the *FANCD2* and *FANCI* orthologs, *fcd-2* and *fnci-1*, has uncovered aberrant pharyngeal pumping and thrashing, suggesting a defect in neuronal circuitry. Analysis of cholinergic (ACh) neurons using the aldicarb assay revealed that *fcd-2* and *fnci-1* animals paralyze at slower rates than wild-type animals under conditions of replication stress, suggesting a defect in ACh motor neuron circuitry. We have also found that tail sensory phasmid neurons of *fcd-2* and *fnci-1* animals are unable to backfill fluorescent dye as efficiently as wild-type animals, suggestive of early-onset neurodegeneration. We have also determined that *fcd-2* and *fnci-1* animals display reduced numbers of ACh and dopaminergic (DA) neurons, compared to wild-type animals.

Taken together, our results indicate an important role for the *C. elegans* FCD-2 and FNCI-1 proteins in nervous system development and maintenance, particularly under conditions of replication stress. Our studies provide greater mechanistic insights into the molecular biology of FANS, a critical step in the discovery of improved diagnostic, preventative, and therapeutic approaches.

695B Catecholamine biosensing in the nIR-II window via functionalized single-wall carbon nanotubes

Rikal Levy[1], Zeinab Yadegari[21]Fisk University, [2]Department of Life and Physical Sciences, Fisk University

Dopamine, a catecholamine neurotransmitter, is implicated in the pathogenesis of numerous diseases and disorders, from schizophrenia & addiction to Parkinson's disease. *Caenorhabditis elegans'* wide use as a model for dopaminergic disorders is limited by current imaging methods that render visualization receptor dependent. Current fluorescent imaging of processes within *Caenorhabditis elegans* takes advantage of their transparency to visualize neuron level processes through fluorescent reporter-receptor tagging. However, this limits visualization to receptor-binding events and overlaps with the organism's intestinal autofluorescence window. To ameliorate this, we intend to validate for use an emerging imaging method of receptor-independent imaging, functionalized single-wall carbon nanotubes (SWCNT). Non-covalent functionalization of SWCNT with ssDNA renders them responsive to dopamine at biologically relevant levels. Upon dopamine exposure, these SWCNT-ssDNA constructs emit fluorescence in the nIR-II window and provide high spatiotemporal resolution outside of *Caenorhabditis elegans*' autofluorescence window. We anticipate our results will allow for receptor independent visualization of dopamine dynamics in real time via nIR-II fluorescence, helping to better elucidate mechanisms underlying dopamine transmission and mechanistic breakdown such as characterization of extrasynaptic dopamine in DAT-1 SWIP phenotypes. Additionally in validating SWCNT use for dopamine imaging, we hope to outline a process that is transferable to other signaling molecules; as in prior literature, SWCNT have been rendered responsive to a multitude of biologically relevant molecules via differential functionalization methods.

696B Transcription factor cooperation and chromatin accessibility in neuronal identity

José Ignacio Jordá-Llorens, Jessica Valdivia, Eduardo Leyva Diaz Instituto de Neurociencias, CSIC-UMH

The complexity of the nervous system stems from the remarkable diversity of its fundamental units, the neurons. Neurons encompass a large variety of information processing and propagating cells with shared features, as well as highly divergent characteristics including morphology and physiology. The identity of each neuronal type is established by unique transcriptional programs during development and actively maintained throughout life. Disruptions in these programs can lead to severe neurodegenerative and neuropsychiatric disorders, such as Alzheimer's and Parkinson's disease. A crucial step in understanding nervous system function is to define the molecular identity of a fully differentiated neuron. Genetic and biochemical studies have identified a conserved set of neuronally expressed proteins, many of which are involved in synaptic vesicle dynamics and are essential for neuron-neuron communication. The genes encoding these proteins form a common molecular toolkit shared by all neurons and are key to defining neuronal identity. Studies in *C. elegans* have shown that the expression of these shared synaptic genes is regulated through the combined action of CUT homeobox transcription factors, which are broadly expressed across the nervous system, and neuron type-specific master regulators known as terminal selector transcription factors.

Previous research has shown that the CUT homeodomain binding motif is strongly associated with differential chromatin accessibility between neurons and fibroblasts, suggesting that CUT factors may function as pioneer factors. Chromatin accessibility plays a critical role in defining cell type identity, as open chromatin regions are highly cell-type specific and closely linked to the binding sites of transcription factors. We propose that CUT factors regulate the binding of additional transcription fractors by modulating chromatin accessibility. To assess the impact of CUT factors on the neuronal chromatin landscape, we have developed a protocol to isolate *C. elegans* neuronal nuclei using FACS, followed by profiling their chromatin accessibility in CUT homeobox gene mutants. These analyses will provide new insights into how CUT factors influence chromatin accessibility and regulate synaptic gene expression and neuronal function.

697B A genetic screen for modulators of stress-induced sleep in *C. elegans* using a robotic worm picking system

Zihao (John) Li[1], Jing Liang[1], Kerry LeCure[2], David Raizen[2], Christopher Fang-Yen[1]1Department of Biomedical Engineering, The Ohio State University, [2]Department of Neurology, University of Pennsylvania

Animals sleep more when they are sick. In *C. elegans*, stress-induced sleep (SIS) is controlled by epidermal growth factor (EGF) activating ALA and RIS neurons, which release multiple neuropeptides to induce sleep. However, the regulatory mechanisms of EGF and the elements acting downstream of ALA/RIS signaling remain poorly understood. To shed light on the mechanisms of SIS, we conducted a genetic screen for modifiers of UV-induced sleep. We used the Million Mutation Project (MMP), a collection of 2007 mutagenized and sequenced strains. The screen requires long-term behavioral assays in clones of animals, making the experiments labor intensive. To enhance throughput, we applied our recently developed worm picking robot to maintain the strain library, conduct sleep assays in the multi-well WorMotel platform, and to carry out other procedures. Aided by robotic automation, our screening throughput was ~50% higher compared to manual methods. Using the robotic system, we identified 3 MMP strains displaying strong deficiency in SIS, out of 190 screened. To identify mutations causing the phenotype, we tasked the robot to perform genetic mapping of the mutations using the sibling subtraction method (SSM). Whole-genome sequence data from SSM indicate multiple novel candidate genes linked to SIS in *C. elegans*. Further exploration of these genes holds potential for enhancing our understanding of the genetic basis of sickness sleep. Moreover, our screening methods serve as a model for expediting other genetic screens through robotic automation.

698B Sensory Integration Across Development Drives Behavioral Sensitization in Male *C. elegans*

Yun-Jung Tu, Kuan-Ting Liu, Yu-Chen Lin, Chun-Hao Chen National Taiwan University

Learning and memory enable animals to predict and adapt their behaviors based on past experiences. Sensitization, a form of non-associative learning, typically amplifies behavioral responses following exposure to a potent stimulus. However, whether animals can integrate multiple sensory experiences to drive sensitization remains unclear. Here, we describe an atypical form of sensitization in male *C. elegans* that enhances reproductive behaviors through the integration of sensory experiences across developmental stages. Specifically, exposure to volatile pheromones, but not ascarosides, reduces the latency of vulva location and spicule protraction during mating. This sensitization occurs independently of immediate feedback from a mate and can be induced by spicule protraction upon aldicarb treatment, even in the absence of partners. Notably, a single pheromonal exposure in either larval or adult stages is insufficient; instead, males must integrate pheromone exposure from both stages within a short time window of less than four hours per experience. This process depends on AWCoff, a functionally asymmetric olfactory neuron, which likely mediates glutamatergic signaling via the GLR-1 glutamate receptor. Additionally, cGMP channels contribute to this form of sensitization. Our findings reveal a novel mechanism of behavioral sensitization in male *C. elegans*, highlighting how early sensory experiences shape learning, memory, and behavioral plasticity in animals.

699B Establishing *C. elegans* nervous system biophysical atlas by systematic electrophysiological recording

Minxian Peng, Shuqi Chen, Qiang Liu City University of Hong Kong

There are many theories about how behavior is controlled by the nervous system. Testing and refining these theories would be greatly facilitated if we could accurately simulate an entire nervous system, replicating brain dynamics in response to various stimuli and contexts. However, modeling a nervous system by reconstructing biological circuits requires understanding each neuron's output/input relationship, which is determined by its intrinsic biophysical properties. Current efforts to model mammalian nervous systems are limited by recordings from only a fraction of their many complex subsystems. To address this challenge, we chose *C. elegans* as our research model. *C. elegans* has a simple and well-defined nervous system consisting of only 302 neurons and a complete connectome, making it possible to uncover the entire circuit at single-cell resolution. Guided by empirical data and the available connectome information in *C. elegans*, we aim to construct a circuit-level model that can simulate information flow, network dynamics, and motor output. To achieve this goal, we plan to systematically record every neuron type in *C. elegans* using our in vivo whole-cell patch-clamp method. Here, we present our progress on systematic electrophysiological recordings from worm neurons. Ultimately, we will collaborate with theorists to construct a conductance-based, system-level model that can help us understand how the brain directs behavior.

700B Effect of psychedelics on locomotion in *C. elegans*

Shawn Lockery, Amanda White, Adele Bauer, Kathy Chicas-Cruz, Serge Faumont University of Oregon

Psychedelics have recently re-emerged as promising treatments for psychiatric conditions such as depression, PTSD, and substance use disorder. A vigorous effort is now underway to improve the efficacy and accessibility of psychedelic therapies. At present, this effort is taking place without a deep understanding of the mode of action of psychedelics. Genetically tractable invertebrate models, such as *Drosophila* and *C. elegans*, with short generation times and a wealth of molecular genetic tools, offer a key adjunct to mode of action studies currently underway in rodents. The first step in establishing these models in psychedelic research is to identify behavioral phenotypes associated with psychedelic exposure.

Classic psychedelics fall into three main types: tryptamines, ergolines, and phenethylamines. Psychedelics are high affinity serotonin receptor agonists. Using locomotion (crawling) as a readout, we screened two tryptamines (dimethyltryptamine (DMT) and psilocin (PSI), the active metabolite of psilocybin), one ergoline (lysergic acid diethylamide (LSD)), and one phenethylamine (2,5-dimethoxy-4-iodoamphetamine (DOI)) for behavioral effects in *C. elegans*. Synchronized young adult worms were exposed to the drug at a concentration of 500 µM in M9 buffer for 30 min, placed individually in 1 cm diameter NGM arenas, then tracked for five minutes. Control worms were exposed to M9 only. Tracking data were analyzed in terms of forward speed and the probability of reverse locomotion. DMT and DOI had no effect on either metric. PSI and LSD significantly increased reversal probability by 28% and 140%, respectively. Neither drug affected forward speed at this dose. We chose LSD for further study because of its dramatic effect on reversal probability. The LSD dose response curve for reversal probability showed no effect at 10 µM but increasing effects at 50 µM, 100 µM, and 500 µM. The dose response curve for forward speed showed a significant reduction at 10 µM, 50 µM, and 500 µM but no effect at 100 µM. Thus, LSD has dose-dependent effects on reversal probability and forward speed.

We conclude that at least two psychedelic drugs have behavioral effects in *C. elegans*. The locomotory slowing we observed in response to LSD could reflect serotonin receptor agonism. As there were no overt sensory stimuli in our experiments, we propose that LSD and PSI increase the probability of spontaneous reversal events. This study suggests that the command neurons for reverse locomotion could be a target of psychedelics in *C. elegans*.

701B Identification of monoaminergic neurons in *Pristionchus pacificus*

Curtis M Loer[1], Luke T Geiger[2], Hyunsoo Yim[2], Yasmin H Ramadan[2], Megan F Hampton[3], Diane V Bernal[3], Heather R Carstensen[3], Steve J Cook[2], Oliver Hobert[2], Ray L Hong[31]Biology, Univ San Diego, [2]Biological Sciences, Columbia Univ, HHMI, [3]Biology, Cal State Univ, Northridge

Nervous systems of many nematodes are remarkably similar despite considerable genomic divergence. To examine nervous system evolution, we are characterizing the *Pristionchus pacificus (Ppa)* nervous system to compare with that of *C. elegans (Cel)* and other nematodes, including the neurotransmitters (NTs) expressed in specific *Ppa* neurons. A small but important subset of neurons in nematodes use monoamine NTs, including serotonin (5HT), dopamine (DA), tyramine (TA) and octopamine (OA). A marker of all monoaminergic (MA) neurons is vesicular monoamine transporter (VMAT) encoded in *Cel* by the *cat-1* gene. To identify MA neurons in *Ppa,* we identified the ortholog *Ppa-cat-1* gene (hence referred to as *cat-1*) and generated a transgenic *cat-1::GFP* reporter in *Ppa*. We further corroborated expression of *cat-1* and other NT-specific marker genes via *in situ* hybridization chain reaction (HCR), including *tph-1* (5HT), *cat-2* (DA), *tdc-1* (TA & OA), and *tbh-1* (OA). Expression of *cat-1* transcripts by HCR was found in all neurons expressing *cat-1::GFP* from the extrachromosomal array (which showed considerable mosaicism); cells marked with GFP were identified by soma position and structure of neurites. As has been reported previously using anti-5HT staining, some 5HT-expressing neurons in *Ppa* are different from those in *Cel*; we confirmed and expanded these identifications to include pharyngeal *I1 & NSM, head *RIP & ADF, and vulval region *VC1-4 (*different from *Cel*). All of these cells also co-expressed *tph-1* and *cat-1* transcripts plus *cat-1::GFP*. There is no 5HT-positive HSN in *Ppa;* we similarly saw no *cat-1* or *tph-1* HCR signal near the vulva. We identified DA (CEPD/V, ADE, PDE), OA (RIC), and TA (RIM) neurons – apparently the same as in *Cel* – all of which co-expressed *cat-1* transcripts and reporter with their respective NT-specific marker genes *cat-2, tbh-1, tdc-1*. There are also as yet unidentified *cat-1* transcript-positive cells in the head, one pair in the dorsal anterior ganglion, and another pair near to CEPD & ADF; these might use an unidentified MA.

702B A microfluidic sorter to separate *Caenorhabditis elegans* by gravitaxis

Hui Ma[1,2], David M Raizen[2], Haim H Bau[11]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, [2]Department of Neurology, Perelman School of Medicine, University of Pennsylvania

Falls among the elderly and individuals with neurodegenerative diseases leads to high medical costs and severe impacts on quality of life. However, the molecules and neural circuits involved in gravity sensing and response remain poorly understood. Identifying genes required for processing gravitational information could inform potential interventions. We are using *C. elegans* as an animal model to understanding gravity response. Previously, we demonstrated that wild-type *C. elegans* can sense and respond to gravity by swimming downwards, whereas the dopamine-deficient *cat-2* mutants are impaired in this behavior. To facilitate the identification of genes required for gravity sensing, we developed a high- throughput system for sorting gravitaxis-impaired from gravitaxis-competent worms. Our device uses a fluidic system with vertical columns filled with a buffer that is slightly denser than the worms. Gravitaxis-deficient worms passively float upwards and are collected at the top, whereas gravitaxis-capable worms swim downward and are collected at the bottom of the device. We designed the sorter and predicted its performance using a theoretical model, and have shown that it is capable of sorting gravitaxis-deficient *cat-2* mutants from wild-type worms.

703B Calcium Imaging in *Pristionchus pacificus* ASE Neurons Reveals Laterally Asymmetric Responses to Ammonium Salts

Marisa Mackie, Ray L Hong Biology, California State University, Northridge

Two distantly related model nematode species *Caenorhabditis elegans* and *Pristionchus pacificus* share the exact same number of homologous amphid neurons but exhibit remarkable differences in their chemosensory profiles, neuron morphology, and ecology. While amphid neurons and their corresponding sensory stimuli are well-described in *C. elegans*, a neuronal map characterizing the various sensory modalities does not yet exist for *P. pacificus*. Therefore, to map chemical stimuli to their cognate amphid neurons in *P. pacificus*, we conducted calcium imaging experiments to measure responses to various attractive salts using *Ppa-che-1p::RCaMP* reporter. We found that the *P. pacificus* ASE neurons show left- and right-specific responses to NH4Cl, NH4I, and NaCl in a concentration-dependent manner. Mutations in the ASER-specific guanylyl cyclase, *Ppa-gcy-22.3*, resulted in loss of NH4Cl calcium response while unexpectedly enhanced attraction in behavioral assays towards NH4Cl but not NH4+ specifically. Surprisingly in *P. pacificus*, *Ppa-che-1* and *Ppa-ttx-1* are co-expressed in the AFD neurons. The putative thermosensory AFD neurons exhibited responses to salts symmetrically but in manners distinct from the ASE neurons: the NaCl response in the AFD was biphasic while the ASER showed an "OFF" response. Knockdown of ASE and AFD neuronal functions using *Ppa-che-1p::HisCl1* abolished attraction towards ammonium salts, although *Ppa-che-1* mutants did not differ in their attraction towards NH4I or NaCl, with less severity than the *Cel-che-1* mutants. Our results are consistent with the model that unlike in *C. elegans, che-1* is not as important for salt sensing, but the *che-1*-expressing ASE and AFD neurons are indispensable for taste reception. Our future directions include investigating the roles of *che-1* and *ttx-1* in temperature sensing by *P. pacificus* ASE and AFD neurons.

704B Nematode behavior in complex, soil-like environments

Yuheng Pan[1], Sima Maleki[2], Adam Smith[1], Zhaochen Yang[1], Hongyi Xiao[1], Eleni Gourgou[1,21]University of Michigan, [2]Wayne State University

Adaptive locomotion allows organisms to thrive in changing environmental conditions. While animals move through their environment, they also reshape it. *C. elegans* nematodes, the natural habitat of which includes muddy soil, display remarkable locomotive adaptability while steered by a compact nervous system. Therefore, they can be highly informative in the effort to identify key neuromechanical requirements for adaptability with sensory feedback in complex and changing environments. We aim to characterize the locomotive behavior and decision-making of wild type and mutant nematode strains upon entering and as they travel through a quasi-two-dimensional layer of disordered and deformable microparticles that resemble the granular texture of soil. We experimentally track the locomotive behavior of *C. elegans* in granular terrains of different properties, and the position of the surrounding particles, using customized deep learning-based algorithms. We show that the packing density of particles as well as prior experience and genetic background of nematodes affect the locomotive behavior of the latter, e.g., their decision to enter the arena, traveling speed, frequency of turns, forward/backward motion ratio, etc. Moreover, particle properties and packing density affect the way moving animals interact with microparticles, e.g., crawling through or over a particle monolayer or opting for low dense areas. In addition, we analyze the impact that moving nematodes have on the granular medium itself, which indicates a continuous, bidirectional interaction. Our work sets the stage for the analysis of nematode behavior in a dynamically changing granular environment, shaped by the animal itself.

705B Modeling the *EBF3* neurodevelopmental syndrome in *C. elegans*

Jessica S Markman[1], Honorine J Destain[2], Jayson J Smith[3], Paschalis Kratsios[31]Biological Science, Neurobiology, University of Chicago, [2]Biological Sciences, Development, Regeneration, and Stem Cell Biology, University of Chicago, [3]Biological Sciences, Neurobiology, University of Chicago

EBF3 Syndrome is a severe neurodevelopmental syndrome caused by mutations in the *EBF3* gene, which belongs to the ancient family of conserved Collier/Olf/EBF (COE) transcription factors, known to play important roles in nervous system development in worms, flies, and mice. The syndrome is characterized by motor developmental delay, ataxia, and intellectual disability. To date, more than 80 *EBF3* mutations have been identified as causal, resulting in a syndrome prevalence of 1 in 1,200 children. However, our understanding of the cellular and molecular basis of the *EBF3* neurodevelopmental syndrome remains poor, in part due to the lack of in vivo animal models. To address this critical need and uncover the mechanisms leading to pathogenicity, I will model 15 of the most common *EBF3* mutations in *C. elegans*, leveraging its powerful genetics and simple nervous system anatomy. The human *EBF3* gene and the *C. elegans* ortholog *unc-3*, a terminal selector essential for motor neuron (MN) identity establishment and maintenance, share 82.1% similarity of their DNA binding domain (DBD). Importantly, not only are the majority of *EBF3* Syndrome mutations found in this DBD region, but so too are those associated with the most severe phenotypes. Therefore, *C. elegans* presents an excellent opportunity to model the human mutations causative for *EBF3* syndrome using its endogenous gene, *unc-3*. This analysis will clarify whether gain- or loss- of function mechanisms are driving syndrome pathogenesis. Specifically, I will use a *gfp* reporter for UNC-3 to assess any changes in protein localization with each mutation. I will also examine any resulting differences in MN identity and in expression of UNC-3 targets. Simultaneously, I will score behavior to determine the severity of each mutation. Using these molecular and behavioral datasets, I may begin to find a pathogenic mechanism common across mutations and/or unique molecular differences associated with symptom severity. This study serves as a key step in increasing our understanding of this neurodevelopmental syndrome and will ultimately pave the way for future work on therapeutics.

706B A genetic toggle switch for touch sensation

Filipe Alberto Gonçalves Marques[1], Yihan Chen[1], Margaux Marinelli[2], Paschalis Kratsios[11]Neurobiology, University of Chicago, [2]University of Chicago

The sense of touch critically depends on the continuous function of specialized mechanosensory circuits, yet the underlying genetic programs remain poorly understood. Here, we report that CFI-1, an ARID3-type transcription factor (TF), functions as a genetic toggle switch for posterior touch sensation in the nematode *C. elegans*. Transient protein depletion during adult life revealed that the presence or absence of CFI-1 generates a digital-like (ON or OFF) effect in the molecular identity program of two lumbar interneuron types (PVC and LUA), impacting information processing within the posterior mechanosensory circuit. The building blocks of the toggle switch are two interconnected network motifs: (a) a positive autoregulatory motif ensures self-sustained CFI-1 expression, and (b) a double-positive feedback loop between CFI-1 and the Hox protein EGL-5 provides robustness of interneuron identity and information processing. Through genetic mutant analysis, auxin-inducible protein degradation, cell-specific RNAi and chromatin immunoprecipitation sequencing (ChIP-Seq), we provide strong evidence that CFI-1 together with EGL-5 function as terminal selectors in PVC and LUA interneurons. That is, CFI-1 and EGL-5 induce during development and maintain through adulthood the transcription of PVC- and LUA-specific terminal identity genes (e.g., ion channels, gap-junctions, neuropeptides and components of neurotransmitter biosynthesis). These findings uncover critical roles for CFI-1/ARID3 and EGL-5/HOX in the later stages of nervous system development and post-embryonic life. Further, they expose a non-canonical function for Hox proteins in maintaining adult neuronal identity, a departure from their traditional roles in early developmental patterning. Given that orthologs of CFI-1 and EGL-5 are expressed in both invertebrate and vertebrate nervous systems, the gene regulatory mechanisms described here for touch interneuron identity could be conserved across species. We postulate that TF-based toggle switches may constitute a general design principle, offering an unprecedented opportunity to manipulate animal behavior by tuning the expression of a single endogenous gene.

707B Fluorescent tagging of SISS-1/EGF to reveal ectodomain shedding

Darlene Mendez, Cheryl Van Buskirk California State University, Northridge

In response to cellular damage, *C. elegans* enters a programmed quiescent state known as stress-induced sleep (SIS), which is mediated by Epidermal Growth Factor Receptor (LET-23/EGFR) activation within sleep-promoting neurons (ALA and RIS). Our behavioral-genetic data indicate that the sleep signal is an EGFR ligand called SISS-1 (stress-induced sleepless) that is shed by the stress-responsive metalloprotease ADM-4. To visualize this signaling event, we have fluorescently tagged the ectodomain of SISS-1 with the goals of tracking its release from cells following exposure to damaging conditions and examining ADM-4 dependence. While fluorescent tags tend to be quenched by the extracellular environment, we can visualize the shed SISS-1 ectodomain by monitoring its uptake by scavenger cells called coelomocytes. Here we present our studies of SISS-1 coelomocyte uptake, which may provide further evidence of a damage-responsive EGF signal that underlies sleep drive.

708B Exploring the molecular and neural basis of gustation in the skin-penetrating, human-parasitic nematode *Strongyloides stercoralis*

Patricia Mendez[1,2], Michelle L. Castelletto[1], Elissa A. Hallem[1,31]Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, [2]Molecular Biology Interdepartmental Ph.D. Program, University of California, Los Angeles, [3]Molecular Biology Institute, University of California, Los Angeles

Strongyloides stercoralis is a skin-penetrating nematode that infects over 600 million individuals worldwide. *S. stercoralis* infects human hosts as developmentally arrested infective third-stage larvae (iL3s). The iL3s are soil-dwelling and use host-emitted sensory cues to actively navigate through the soil in search of a host to invade via skin penetration. Despite their global prevalence, our understanding of how skin-penetrating nematodes, such as *S. stercoralis,* locate and invade their hosts is very limited. We are investigating how gustation contributes to host invasion in *S. stercoralis* iL3s. We first examined the behavioral responses of *S. stercoralis* iL3s to human sweat and a physiologically relevant concentration of NaCl found in human sweat. We found that human sweat triggers iL3s to disperse, a behavior that may contribute to host invasion by increasing the likelihood of encountering a suitable entry point on host skin. In addition, using an *ex vivo* skin penetration assay, we found that iL3s penetrate rat skin more rapidly in the presence of either human sweat or NaCl. Our results suggest that human-associated gustatory cues stimulate host-invading behaviors. We are now investigating the gustatory neurons and receptors that mediate these responses. In *Caenorhabditis elegans*, salt chemosensation is mediated by members of a large family of receptor guanylate cyclases (rGCs). In *S. stercoralis,* the rGC family contains a one-to-one homolog of a broad salt-sensing receptor in *C. elegans*, *Ce*-GCY-22. We generated a transcriptional reporter for *Ss-gcy-22* and found that it appears to be expressed in a single head neuron. We also generated a transcriptional reporter for the *S. stercoralis* homolog of the *C. elegans* ASE marker gene *Ce-che-1*. We found that *Ss-che-1* co-localizes with *Ss-gcy-22*, suggesting that *Ss-gcy-22* is expressed in one of the putative *S. stercoralis* ASE neurons. Furthermore, the *S. stercoralis* rGC family contains an expansion of rGCs, one of which expresses in the putative ASE neuron opposite to that expressing *Ss-gcy-22*. We are now examining the functional properties of these putative *S. stercoralis* ASE neurons via calcium imaging and investigating their role in regulating gustatory-driven, host-invading behaviors. Our results will provide insight into how the gustatory system of *S. stercoralis* contributes to its ability to invade human hosts, with broad implications for nematode control.

709B Characterization of Neuropeptide Expression Strains

Daviana L. Menendez Escalera[1], Carrie Sheeler[1], Ashley Frakes[1,21]Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, [2]National Institutes of Neurological Disorders and Stroke

Astrocyte-like glial cells act as central regulators of organismal protein homeostasis and longevity in C. elegans. Activation of the unfolded protein response in glia (glial::UPRER) confers a protective effect against ER stress, extending lifespan, and promoting protein homeostasis. Further work demonstrated that these cells coordinate non-autonomous UPRER activation via neuropeptide signaling. Neuropeptides are small peptides that bind G protein coupled receptors to regulate important biological processes including development, behavior, metabolism and aging. To identify the peptides required for initiating cell non-autonomous UPR activation, we performed peptidomics in glial::UPRER animals. This identified a significant increase in the abundance of 8 neuropeptides. However, little is known about the expression profiles of many of these neuropeptides. We generated reporter arrays for each of these specific neuropeptides to determine their cellular sources in vivo. Worms were injected with a plasmid construct that drives the expression of the red fluorescent protein wrmScarlet under the control of the neuropeptide's native promoter. We determined the source of neuropeptides by performing fluorescent microscopy of each reporter strain at Day 1 of adulthood. To identify the specific cell types with wrmScarlet expression, I cross-referenced single cell sequencing data (from both in-house and The *C. elegans* Neuronal Gene Expression Map & Network, CeNGEN) with existing literature and WormAtlas. Neuropeptide expression patterns in these reporter strains were consistent with expected cell types from our single cell sequencing and CeNGEN's dataset. Interestingly, we observed neuropeptide expression in tissues not predicted, or with lower transcriptional counts in our scRNA-seq dataset. In summary, this work validates expected neuropeptide expression patterns, which suggests a role for neuropeptide signaling in these tissues to regulate important biological processes.

710B CAN as a Regulator of Osmotic Homeostasis

Daniel Merritt, Oliver Hobert Biological Sciences, Columbia University

Of the 302 neurons in *Caenorhabditis elegans*, only 3 are required for life: M4, which controls peristalsis of the pharyngeal isthmus, and the bilaterally paired CANL and CANR neurons, which have long processes extending along the excretory canal cell but which have unknown functions. Recently, we reported expression of the vesicular monoamine transmitter *cat-1* in CAN, as well as *snf-3*, a transporter for the non-canonical amino acid neurotransmitter betaine. We have now analyzed the expression pattern of a variety of neuron-specific genes, and found that most, but not all, are expressed in CANs.

Experiments using laser ablation to selectively kill CANs embryonically have shown that animals lacking them die as clear, arrested L1 larvae, but suggested that CAN neurons are dispensable during adulthood. It has previously been suggested that CANs serve to regulate homeostasis of organismal osmolarity by controlling the excretory system, and that this underlies their requirement in the animal, however efforts to understand the role of CAN have been constrained by technical limitations, including very limited throughput and uncertain damage to cells proximate to CAN. To overcome these limitations, we have characterized the effect of CAN ablation in large numbers of worms at different stages of *C. elegans* using an inducible CAN ablation strain, allowing for highly specific ablation and identification of phenotypes with incomplete penetrance. Our findings demonstrate that CAN ablation in most larval stages results in clear, developmentally arrested animals, however we find that it is dispensable during dauer arrest. Contrary to prior reports, we find that CAN plays a crucial role during adulthood in preventing rupture and subsequent expulsion of the intestine through the vulva: a phenotype previously reported in genetically sensitized animals subjected to osmotic stress. Further, evidence across several developmental stages supports a role for CAN in molting, and in particular, in allowing for the successful completion of ecdysis. We are presently conducting transcriptomic and mass spectrometry analysis of CAN-ablated animals to better understand the signaling pathway or pathways through which CAN acts to mediate these phenotypes.

711B Building axons with the NF-Y complex

Pedro Moreira[1], Paul Papatheodorou[2], Roger Pocock[11]Anatomy and Development Biology, Monash University, [2]Deakin University

Establishment of neuronal circuits during development is an exceptionally complex process requiring precisely controlled gene expression. While neurite guidance mechanisms have been the subject of extensive focus over the last four decades, the molecular programs underpinning neurite guidance and termination are not fully understood.

We performed an unbiased forward genetic screen that identified the NFYA-1 transcription factor as a key regulator of neuron fate and axon guidance. Nuclear Factor Y (NFY) is a highly conserved ubiquitous heterotrimeric complex, composed by three subunits NFYA, NFYB and NFYC, which binds to cis-regulatory motifs to regulate target gene expression. Here, we show that NFYA-1 controls neurite guidance of multiple neuronal subtypes. We use the NSM neurons as a model to dissect how NFYA-1 regulates molecular programs during neurite guidance and termination. Our data reveal that NFYA-1 functions cell autonomously to control NSM dorsal neurite development. In addition, using genetic epistasis experiments we delineated how NFYA-1 interacts with known axon guidance pathways to control NSM development.

712B Mapping neuropeptide function – One neuron at the time

Pedro Moreira, Yiwei Zhou, Roger Pocock Anatomy and Development Biology, Monash University

During development, neurons form intricate connectivity networks that enable local and long-range communication throughout the body. These communication networks use ancient signaling molecules called neurotransmitters and neuropeptides to regulate multiple aspects of animal life, including learning, memory, sleep, locomotion, and metabolism. Great strides have been made to fully map the synaptic connectome in different organisms and to understand how neurons use neurotransmitters to communicate. In contrast, understanding of how individual neurons use neuropeptide "cocktails" to modulate animal behavior and physiology is still in its infancy. Neuropeptides are small amino acid chains that can function "wirelessly" and over long distances to control bodily functions. A critical control step in neuropeptide function is their release from dense core vesicles, which is dependent on the conserved calcium-dependent activator protein for secretion (CAPS) — UNC-31 in *C. elegans*.

Here, we describe our generation of a neuron-specific neuropeptide depletion toolkit. The toolkit uses auxin-inducible degradation of the UNC-31 protein to enable spatiotemporal control of neuropeptide release from each individual *C. elegans* neuron. This library of depletion strains will enable us and others to investigate how each neuron uses specific neuropeptide "cocktails" to control animal behavior and physiology. We have extensively validated the toolkit by phenocopying neuropeptide-dependent functions in controlling metabolism and motility from multiple neurons.

713B Establishing a Chronic Cocaine Paradigm in *C.elegans*

Prince Nyree Mosley, Audrey Nyree Gilbert, Shelby N Lauzon, HaoSheng Sun CDIB, The University of Alabama at Birmingham

Cocaine Use Disorder (CUD) is a prevalent problem in American society, affecting over one million people annually and lacking effective therapeutics. One prevalent hypothesis is that chronic cocaine exposure leads to long-lasting molecular and functional changes in the nervous system, which in vulnerable individuals result in behavioral changes that further predispose to loss of control and progression of the disease. Here, we aim to establish *C.elegans* as a novel model to uncover the mechanisms underlying long-lasting neuronal changes in response to chronic cocaine exposure. First, we are developing a chronic cocaine dosing paradigm that results in long-lasting behavioral adaptations. We are examining various locomotive parameters and pharyngeal activity, as these easily quantifiable behaviors allow for high-throughput mechanistic screens. Concurrently, we are examining whether these dosing paradigms have long-lasting inter- and trans-generational effects. The long-term goal of the study is to establish a novel platform where we can readily identify and causally implicate conserved genetic susceptibility loci and molecular regulators that underlie cocaine-induced neuronal plasticity, eventually leading to an improved mechanistic understanding of CUD neurobiology and the identification of novel avenues of therapeutic targets.

714B Understanding how the vulval muscles are mechanically activated in *C. elegans*

Srishti Mullick, Kevin Collins University of Miami

Internal sensory cues such as an internal mechanosensory feedback in the form of stretch, touch or pressure are important signals that drive several involuntary functions. We have been using *C. elegans* to study how mechanosensory feedback regulates egg-laying and other reproductive behaviors. Previous work from our lab has shown that microinjection, generally used to generate transgenic strains, into the gonad of *C. elegans* drives vulval muscle Ca2+ activity, contraction, and egg release. We proposed that acute microinjection mimics the normal increase in stretch that accompanies egg accumulation in the uterus with the vulval muscles being the primary responders to this stimulus. An alternate way to mechanically activate the vulval muscles is by directly prodding them using a glass probe. This triggers a robust Ca2+ transient activity, suggesting these cells respond directly to mechanical feedback. Using this prodding approach and various mutant animals lacking defined mechanosensory pathways, I seek to define the channels and signaling molecules that drive vulval muscle mechanical activation. Prodding of animals lacking synaptic transmission from the HSN egg-laying command neurons and the Ventral C motor neurons show robust vulval muscle Ca2+ activity, suggesting these cells are not mediating this response. Similarly, *unc-13* or *unc-31* mutants lacking small clear vesicle or dense core vesicle transmission show normal vulval muscle prodding responses. These results suggest the vulval muscle prodding responses are direct and likely independent of presynaptic input. Prior gene expression studies have shown that the vulval muscles express several mechanosensory channels including PEZO-1, TMC-1/2, DEG/ENaC channels, and a subset of TRP channels. L-type Ca2+ channels also regulate vulval muscle excitability and egg laying. Using genetic and pharmacological approaches, I have found that TRP-1, TMC-1/2, and L-type Ca2+ channels, but not PEZO-1, facilitate the vulval muscle prodding response. Additionally, I am also presently testing other vulval muscle-expressed mechanosensory channels. Thus, a complete understanding of how mechanosensory channels mediate the prodding response in the vulval muscles may reveal the cellular and molecular mechanisms through which the vulval muscles detect egg accumulation and stretch.

715B Exploring the Distinct Roles of CNG and L-VGCC Regeneration Pathways by Pharmacology

Emma C Nace[1], Lynn Pham[1], Noa W.F. Grooms[2], Samuel H Chung[21]Biology, Northeastern University, [2]Bioengineering, Northeastern University

The mammalian central nervous system exhibits limited regenerative capacity. However, injury to the peripheral sensory axon can induce transcriptional changes that profoundly enhance regenerative potential—a process known as lesion conditioning. We recently identified CREB (*crh-1*) as a key regulator of this process and a mediator of regeneration-associated gene (RAG) transcription. However, the upstream signals governing CREB modulation remain unclear. To further elucidate how CREB signaling drives neuronal repair, we investigated the role of the cyclic nucleotide-gated (CNG) channel *tax-2* and the L-type voltage-gated calcium channel (L-VGCC) *egl-19* in axonal regeneration. We pharmacologically manipulate these pathways in the ASJ neuron using L-cis-diltiazem (LCD), a CNG channel blocker, and Nemadipine-A, an L-VGCC inhibitor. Acute LCD treatment (exposure post-injury) and chronic LCD treatment (continuous pre- and post-injury exposure) produced distinct outcomes, with chronic exposure significantly enhancing ectopic outgrowth compared to acute treatment. Additionally, expression of thioredoxin, a RAG, was markedly increased in this background. Despite these results in the ASJ, behavioral and developmental assays of *tax-2* function in other neurons showed no difference between LCD-treated and control animals, suggesting that *tax-2* inhibition by LCD may vary across different neuronal types.

To explore calcium signaling, we assess the effects of L-VGCC inhibition on regeneration. Consistent with previous findings, blocking *egl-19* activity enhances regrowth, reinforcing the role of calcium influx in restricting conditioning. Notably, LCD treatment does not replicate the phenotypes observed with *egl-19* inhibition or mutation, suggesting that LCD does not strongly inhibit L-VGCC in C. elegans, similar to its specificity in mammals.

Given that CREB activation in mammalian systems is regulated by phosphorylation at distinct sites, our ongoing research explores how these secondary messenger pathways influence CREB activity in lesion conditioning. By advancing our understanding of these mechanism, our findings may inform the development of targeted neuroregenerative therapies.

716B A multilayered gap-junction network is essential for social decision-making

Airi Nakayama[1], Hiroo Kuroyanagi[1], Hironori Matsuyama[2], Ikue Mori[2], Shunji Nakano[11]Department of Biological Science, Nagoya University, [2]Neuroscience Institute, Nagoya University

Early-life social experiences can shape adult behavior. A classic example is density-dependent dispersal behavior, in which animals in small groups tend to exploit local resources, whereas those in high density populations are more likely to explore new habitats. Here, we show that *C. elegans* larvae reared at high density develop into adults that exhibit exploratory behavior in the associative-learning paradigm known as thermotaxis. While animals grown at low density prefer the temperature previously associated with food, those exposed to high density during larval stages instead disperse from this temperature. This modulation is mediated by nematode pheromones, as *daf-22* mutants, which lack an enzyme required for pheromone synthesis, are insensitive to high density and prefer the cultivation temperature. Analysis of temperature-evoked neural activity reveal that the high-density experience alters their perception of thermal valence, a process that requires a gap-junction network spanning multiple neural circuit layers. This network connects pheromone-sensing chemosensory neurons to the circuit that processes temperature information. Furthermore, these gap-junction components also control the decision to enter developmental diapause in response to high density. Our results suggest that the multilayered gap-junction network enables early-life social experience to influence adult behavior and is essential for developmental and behavioral plasticity in social contexts.

717B A hyperpolarizing neuron recruits undocked innexin hemichannels to transmit neural information during *C. elegans* thermotaxis

Airi Nakayama[1], Masakatsu Watanabe[1,2], Riku Yamashiro[1], Hiroo Kuroyanagi[1], Hironori J Matsuyama[1], Atsunori Oshima[1,3], Ikue Mori[1,4], Shunji Nakano[1]1Nagoya University, [2]Osaka University, [3]Gifu University Institute for Advanced Study, [4]Chinese Institute for Brain Research

While depolarization of neuronal membrane is known to evoke the neurotransmitter release from synaptic vesicles, hyperpolarization is regarded as a resting state of chemical neurotransmission. During *C. elegans* thermotaxis, AFD thermosensory neurons, the pivotal neurons for thermotaxis regulation, depolarize in response to warming stimuli and control the activity of their post chemical synaptic partner, the AIY interneuron. Previous studies also demonstrated that cooling stimuli hyperpolarized the AFD membrane potentials and that AFD is indispensable for the behavioral control upon temperature cooling. However, how hyperpolarizing AFD transmit neural information and control the neural circuitry to generate appropriate behaviors remains elusive. Here we report that hyperpolarizing AFD employ hemichannels, the undocked form of gap junction channels, to signal temperature information to AIY.

We conducted a genome-wide survey of innexin genes and showed that UNC-7, a member of innexin family, is required for the regulation of thermotaxis. Innexins are components of hemichannels, which, through docking with other hemichannels, form gap junctions. To address which type of channel is essential for thermotaxis, we generated a chimeric UNC-7 that is predicted to lose the ability to form gap junction but retain hemichannel activity. This chimeric UNC-7 rescued the thermotaxis defect caused by knocking out *unc-7* specifically in AFD, suggesting that UNC-7 acts as a hemichannel to regulate thermotaxis. Calcium imaging showed that *unc-7* mutations did not affect temperature-evoked calcium response in AFD, indicating that UNC-7 functions in a process downstream of calcium influx. To assess whether UNC-7 hemichannels regulate the neuronal activity of AIY, we analyzed neural calcium dynamics of AIY in freely behaving animals and found that UNC-7 hemichannels inhibit the AIY neuronal activity upon membrane hyperpolarization of AFD. We also addressed whether UNC-7 controls known synaptic transmission from AFD. Previous studies showed that the neurotransmission from AFD to AIY is mediated by glutamatergic and peptidergic signaling. To ask whether UNC-7 is involved in these chemical synaptic transmissions, we investigated epistasis between *unc-7* and *eat-4* or *unc-31,* each of which is known to play a role in glutamate or neuropeptide release, respectively. We observed that knocking out of *unc-7* in AFDaffected thermotaxis in strains lacking *eat-4* or *unc-31*, suggesting that UNC-7 controls thermotaxis behavior independently of the chemical transmission from AFD.

Our findings suggest that innexin hemichannels mediate neurotransmission from hyperpolarizing neurons in a manner that is distinct from the synaptic transmission. We speculate that such hemichannels act as calcium-independent regulators of neurotransmission, expanding the way of neural circuitry operations.

718B Synergistic interaction of the homeobox gene *ceh-36* with the terminal selector *che-1* in long-term maintenance of ASE neuron fate

Ananya Natarajan[1], Manon Vleeming[2], Gert Jansen[2], Jeroen van Zon[1]1Autonomous Matter, AMOLF, [2]Developmental Biology, Erasmus MC

Once differentiated, neurons remain in place throughout the organism's entire life. Yet, the mechanisms governing long-term maintenance of cell fate are poorly understood. The genetic networks underlying cell fate often contain positive feedback loops that suggest that they act as bistable switches. While this bistability explains both rapid fate induction and long-term maintenance, it implies that cell fate remains inherently reversible and thus raises the question how spontaneous cell fate loss is avoided. Previously, we showed that the network controlling ASE neuron fate in C. elegans forms a bistable switch, that can be permanently switched off by transient depletion of the master regulator CHE-1. We also identified a potential mechanism, target reservoir buffering (TRB), that explains the lack of spontaneous reversals of ASE fate. Key to TRB is that CHE-1 binds its own promoter with much higher affinity than promoters of its other targets, but how this preferential binding is controlled is an open question.

We found a homeodomain binding site in the che-1 promoter that causes stochastic ASE fate loss when deleted, suggesting a possible homeodomain transcription factor (HD-TF) functions as CHE-1 cofactor in ASE fate maintenance. We screened mutants of all HD-TFs expressed in both ASE neurons, and found that ceh-36(0) mutants progressively lost che-1 expression and ASE function after hatching. Simulations of a model that includes cooperative interactions between CHE-1 and CEH-36 on the che-1 promoter show that such cooperativity strongly inhibits spontaneous fate loss. The model also predicts that stability of the CHE-1 switch is particularly strong if CEH-36 protein turnover is slower than that of CHE-1.

We are testing these model predictions by auxin-mediated CHE-1 and CEH-36 depletions and measuring the impact on CHE-1 and CEH-36 mRNA and protein levels, and on ASE function. Behavioral assays showed that, while salt sensing and hence ASE function was completely abrogated during 24 hrs transient CHE-1 depletion, it fully recovered once CHE-1 depletion was removed. In contrast, we found that transient depletion of both CHE-1 and CEH-36 caused permanent loss of ASE function. This was striking, as depletion of CEH-36 alone hardly impacted ASE function, highlighting the strong synergistic effects of CHE-1 and CEH-36, as also predicted by our model. Overall, these results suggest an important role for HD-TF co-factors in long-term cell fate maintenance.

719B Functional classification of *GNAI1* disorder variants in *C. elegans* uncovers conserved and cell-specific mechanisms of dysfunction

Inna Nechipurenko, Rehab Salama, Eric Peet, Logan Morrione Biology and Biotechnology, Worcester Polytechnic Institute

Neurodevelopmental disorders (NDDs) comprise a range of conditions that commonly include intellectual and learning disabilities, autism, attention-deficit/hyperactivity disorder (ADHD), and impairment of sensory modalities. Variants in the human *GNAI1* gene, which encodes Gαi1 subunit of the heterotrimeric G(αβγ) proteins were recently identified as a cause of a novel NDD characterized by developmental delay, hypotonia, seizures and/or autistic features; however, the functional consequences of these variants remain unknown.

Knockdown of *GNAI1* in human cells interferes with the assembly of primary cilia – specialized signaling compartments that play critical roles in neuronal development. Similarly, *C. elegans* Gαi/o-like ODR-3, which exhibits 48% sequence identity and 66% similarity to Gαi1, shapes cilia morphology in select amphid neurons. Here, we leveraged the well-defined genetics of *odr-3* together with the robust cellular phenotypes of *odr-3(lf)* mutants to functionally classify seven patient *GNAI1* variants that map to conserved, identical residues in *C. elegans* ODR-3. We find that all examined patient variants differentially disrupt ODR-3 sub-cellular localization and cilia morphology. Interestingly, although all examined homozygous variants exhibit morphologically defective AWC cilia, only *A328P* mutation in the conserved nucleotide-binding motif impairs AWC-mediated chemotaxis toward benzaldehyde, in line with a previously reported divergence of ODR-3 function in AWC ciliogenesis and chemotaxis. In contrast, a subset of the examined variants that preclude ODR-3 from localizing to cilia in ASH neurons, also impair ASH-mediated glycerol avoidance, suggesting cell-specific differences in the functional impact of the examined patient variants.

Finally, experiments in human cells confirmed that the same subset of NDD variants that preclude ODR-3 trafficking to the cilium in *C. elegans* neurons also disrupts ciliary localization of human Gαi1. Overall, this study provides new insight into the conserved and cell-specific cellular mechanisms that are disrupted by the *GNAI1* patient variants and establishes a genetic pipeline for rapid functional classification of NDD-associated mutations.

720B Polycomb Repressive Complex 1 deposits histone H2AK119ub to regulate gene expression, neuronal migration, and behaviour

Jevithen Nehru, Kailynn MacGillivray, Sofia Eiras, Arneet Saltzman Cell & Systems Biology, University of Toronto

The PRC1 complex deposits histone H2AK119ub to regulate gene expression and plays conserved roles in neuronal development, from *C. elegans* to mammals. The *C. elegans* PRC1 complex components *mig-32* and *spat-3* have reported roles in neuronal cell fate specification, migration and axon guidance, but the mechanisms involved are not well understood. Here we investigate the spatiotemporal requirements and genetic interactors of *mig-32* and *spat-3* in hermaphrodite-specific neuron (HSN) migration and PVQ interneuron axon guidance. To test if PRC1 acts autonomously within neuronal tissue, we employed complementary tissue-specific transgene addback and auxin-inducible degron approaches. Ubiquitous but not pan-neuronal *mig-32* transgene expression rescued HSN migration in *mig-32* mutants. Similarly, ubiquitous but not tissue-specific auxin-induced depletion of SPAT-3 phenocopied *spat-3* null mutants. While these results are consistent with nonautonomous PRC1 action, we cannot rule whether our transgenes and TIR-1 constructs are not expressed sufficiently early during embryogenesis. Indeed, temporally-controlled auxin-mediated depletion of *spat-3* indicates that it acts during embryonic stages, when HSN migration takes place. To understand the pathways through which PRC1 regulates neuronal development, we are using a genetic epistasis approach. These experiments suggest that PRC1 likely acts in the same pathway as *set-2*, an H3K4 histone methyltransferase, to regulate HSN and PVQ axon guidance. In addition, double mutants with *mig-32* and *daf-16*, a FOXO homolog, reveal a strong enhancement of the HSN migration phenotype, supporting a model where PRC1 must operate in parallel with other distinct pathways to coordinate neuronal development. To test the impact of PRC1 on nervous system function, we also performed locomotion and egg-laying assays. PRC1 mutant worms show a reduced thrashing rate in liquid and display abnormal movements more often. On solid media, mutants explore a smaller fraction of their environment. Additionally, the egg-laying circuitry is partially defective, as PRC1 mutants lay fewer eggs and have weaker responses to drugs known to trigger this behaviour. Our data so far suggest that PRC1 collaborates with multiple pathways across cell types to regulate neuronal development and function.

721B Involvement of CaMKIIγ in Learning and Memory through Behavior and GLR-1 Glutamate Receptor Expression

Kevin J Nelson[1], Aashir Ahmed[2], Justin O King[2], Aicha B Tokar Falatah[2], Jacqueline K Rose[21]Biology, Western Washington University, [2]Psychology, Western Washington University

Calcium/calmodulin-kinase II (CaMKII) plays a role in neuronal mechanisms of learning and memory. In *C. elegans*, the *unc-43* gene is an ortholog of CaMKII with *C. elegans* isoform sequences aligning to mammalian CaMKIIδ or CaMKIIγ. A mutation that affects expression of all UNC-43 isoforms (i.e., *unc-43*(n498)) modulates glutamate receptor expression (specifically GLR-1) in neurons; however, behavioral studies of learning with this strain are limited due to the *unc*oordinated motor phenotype. The *unc-43*(gk452) mutant strain is unique to other *unc-43* mutants as it is superficially wild-type; thus, allowing for behavioral studies of learning and memory. Interestingly, the *unc-43*(gk452) mutation affects the coding region of UNC-43, isoform t, for which the protein sequence is more than 40% identical to CaMKIIγ. Previous studies report that expression of *unc-43*(gk452) regulates learning pathways by activating cAMP-response element binding (CREB) protein. The current study examines the *unc-43(*gk452*)* strain using a learning protocol where conditioning is restricted to a brief, discrete time period. Based on signaling competition, this learning assay described previously by our lab employs pairing two stimuli that drive opposing motor responses: blue light ~480 nm elicits forward locomotion while a mild mechanosensory vibration results in backward locomotion. Learning and memory in *unc-43*(gk452) mutant worms was tested at 1-, 10-, or 60 minutes after one block of 5 stimulus pairings. Initial data with this protocol indicates a deficit in learning after one block of training measured at 10 minutes. To examine the effect of *unc-43(gk452)* on GLR-1 glutamate receptor expression, confocal imaging of an *unc-43(gk452)*; GLR-1::GFP cross was performed. A full knockout of unc-43, *unc-43(js125),* was imaged for a comparison with *unc-43(gk452)*. Observing learning and memory, as well as receptor expression in intact animals, we will be able to further describe the role of CaMKIIγ in associative learning and memory.

722B The role of tomosyn in alcohol-modulated habituation in *Caenorhabditis elegans*

Yi Qing Yvette Ni, Conny Lin, Catharine H Rankin The University of British Columbia

Habituation is a fundamental form of learning observed across species, characterized by the progressive decrement of behavioural responses to repeated stimuli. Disruptions in habituation mechanisms have been linked to various neuropsychiatric conditions, and identifying the key molecular pathways underlying these processes remains an ongoing effort. In this study, we examined the role of *tom-1*, which encodes tomosyn, an inhibitory protein that controls synaptic transmission. This is the first work to implicate tomosyn in the effects of acute ethanol exposure behaviour in *Caenorhabditis elegans*. A possible mechanism of habituation is modulation of synaptic release, and we hypothesized that alcohol might alter synaptic release; if so, *tom-1* may play a role in that mediation. We assessed habituation at a 10 s ISI in the well-characterized tap withdrawal response. We found that in the absence of ethanol, *tom-1* mutants showed very similar habituation to wild-type worms, indicating that *tom-1* does not play a role in normal habituation. On 400mM ethanol, wild-type worms showed much shallower habituation for response probability than worms off of ethanol. In contrast, the habituation of reversal probability in *tom-1* mutants was not altered by ethanol. These findings suggest that tomosyn is required explicitly for ethanol's effects on habituation of response probability for the tap-withdrawal response. Our next step in determining where *tom-1* mediates ethanol's effects on habituation of response probability is to do extrachromosomal rescue experiments restoring *tom-1* function both pan-neuronally and specifically within the mechanosensory and command neurons of the tap-withdrawal circuit. This work provides insights into the genetic and neurobiological basis of habituation and highlights potential molecular targets for understanding ethanol-induced behavioural plasticity.

723B A nuclear hormone receptor *nhr-76* programs age-dependent chemotaxis decline

Rikuou Yokosawa, Kentaro Noma Graduate School of Science, Nagoya University

Active genetic mechanisms that cause aging of the nervous system are largely unexplored. To examine the genetic mechanism of age-dependent behavioral decline, we used chemotaxis behavior in *C. elegans*. On day 1 of adulthood, animals show robust chemotaxis behavior toward an attractive volatile odorant, diacetyl. On the other hand, post-reproductive day 5 adults do not. Through a novel forward genetic screen, we identified the gene encoding a nuclear hormone receptor, *nhr-76*, whose loss-of-function mutants ameliorate the age-dependent chemotaxis decline. We found that NHR-76 functions in the AWA sensory neurons and cell-autonomously downregulates the expression of *odr-10* encoding the diacetyl receptor in an age-dependent manner. The activity of nuclear hormone receptors can be altered by their hydrophobic ligands, such as steroid hormones and vitamin D. Because NHR-76 expression and localization in the AWA sensory neurons did not change during aging, the presence of its hydrophobic ligands might alter the activity of NHR-76 to cause age-dependent chemotaxis decline. In contrast to the chemotaxis toward diacetyl, *nhr-76* mutants did not ameliorate the chemotaxis toward other odorants, such as pyrazine and benzaldehyde. Therefore, *nhr-76* might convert the systemic aging signal to the specific behavior. Our findings imply that post-reproductive behavioral decline can be programmed by an active genetic program.

724B A cytidine deaminase regulates axon regeneration by modulating the functions of the *Caenorhabditis elegans* HGF/plasminogen family protein SVH-1

Takafumi Nomachi[1], Tatsuhiro Shimizu[2], Kunihiro Matsumoto[1], Naoki Hisamoto[11]Nagoya University, [2]Kobe University

The pathway for axon regeneration in *Caenorhabditis elegans* is activated by SVH-1, a growth factor belonging to the HGF/plasminogen family. SVH-1 is a dual-function factor that acts as an HGF-like growth factor to promote axon regeneration and as a protease to regulate early development. It is important to understand how SVH-1 is converted from a protease to a growth factor for axon regeneration. In this study, we demonstrate that cytidine deaminase (CDD) SVH-17/CDD-2 plays a role in the functional conversion of SVH-1. We find that the codon exchange of His-755 to Tyr in the Asp–His–Ser catalytic triad of SVH-1 can suppress the cdd-2 defect in axon regeneration. Furthermore, the stem hairpin structure around the His-755 site in *svh-1* mRNA is required for the activation of axon regeneration by SVH-1. These results suggest that CDD-2 promotes axon regeneration by transforming the function of SVH-1 from a protease to a growth factor through modification of *svh-1* mRNA.

725B Post-training sleep and olfactory synapses are impacted in aged *C. elegans* with declining long-term memory

Kateryna Tokalenko[1], Hazel Guillen[1], Vanessa Garcia[1], Emma Odisho[1], Andrew Bykov[1], Emily SooHoo[1], Price Pettie[1], Mariam Mortada[1], Sukhdeep Kaur[1], Maleiyah Harris[1], Annabel Nguyen[1], Gabriel Hertel[1], Martina Bremer[1], Miri Van-Hoven[1], Noelle L'Etoile[21]San Jose State University, [2]UC San Francisco

Increasing evidence suggests that sleep loss contributes to age-related memory decline. While sleep is known to enhance memory consolidation, less is understood about how aging affects the relationship between sleep and memory formation. Our goal is to use C. elegans, a nematode capable of long-term olfactory memory formation and rapid aging, to test our hypothesis that post-training sleep and olfactory synapses are impacted in aging animals that have impaired long-term memory. C. elegans are innately attracted to butanone, and if day 1 adults are trained with three cycles of exposure to this odorant without food, then allowed to sleep after training, they can learn to ignore this odor and remember their training for more than 16 hours. However, if aged animals, for example day 3 adults, receive the same training, they initially learn to ignore butanone, but fail to form a long-term (16 hour) memory. To determine whether post-training sleep is impacted in aged animals, we assessed two key sleep characteristics: feeding and arousal latency. In day 1 adults, feeding rate, assessed by quantifying pharyngeal pumping, was significantly reduced in trained animals during the period of post-training sleep. Similarly, we found that arousal latency, assessed by measuring the time to complete a forward body bend after a tail touch with an eyebrow hair, was significantly higher in trained animals during the period of post-training sleep. However, we found that in aged animals, neither feeding rate nor arousal latency were significantly different from naive aged animals, consistent with reduced quality post-training sleep. To determine if olfactory synapses were impacted in aging animals, we used the fluorescent split GFP-based trans-synaptic marker Neuroligin 1 GFP Reconstitution Across Synaptic Partners (NLG-1 GRASP) to examine synapses between the butanone-sensing AWC neurons and their primary postsynaptic partners, the AIY interneurons. We found that these olfactory synapses are significantly reduced between day 1 and day 3 of adulthood, which may affect long-term memory consolidation. We hope these findings aid in understanding how aging impacts the ability of sleep to promote long-term memory formation, and help pave the way to develop more effective therapeutics to treat forms of dementia that are exacerbated by lack of sleep. This work is supported by the NIH (AG091327 and NS087544 to MV and NL).

726B A neuropeptide signaling pathway mediates pheromone avoidance behavior in *C. elegans*

Eujeong Oh[1], Hyeonjeong Hwang[2], Kyuhyung Kim[21]Brain Sciences, Daegu Gyeongbuk Institute of Science and Technology, [2]Daegu Gyeongbuk Institute of Science and Technology

Animals adjust their behavior to environmental changes to improve their chances of survival. Despite the crucial role of neuropeptides in modulating behavior, the neuronal and molecular mechanisms underlying neuropeptide mediated behavioral plasticity are not fully understood. *C. elegans* secretes a pheromone mixture called ascarosides. A specific pheromone component, ascr#3, elicits repulsion in wild-type hermaphrodites. This response is further modulated by sex, stress, and early experience (Jang et al., 2012, Hong et al., 2017, Ryu et al., 2018). Previously, we have shown that a *flp-26* regulates ascr#3 avoidance behavior (Hwang et al., in prep). To further understand the mechanism of *flp-26*-mediated avoidance, we first aim to identify its receptor(s). To this end, we compiled a list of 44 putative GPCR candidates of which expression in ADL was identified from the CENGEN database based on the gene expression levels of single-cell RNA-Seq data. Additionally, NPR-6, DMSR-1, and DMSR-7 were identified as FLP-26 receptors through in vitro studies (Isabel et al., 2023). We are currently investigating their roles in acute ascr#3 responses. We found that *npr-1, npr-20, npr-26, frpr-16,* and *frpr-18* mutants exhibit reduced avoidance. Next, we are currently investigating the expression pattern of these genes in order to determine the expression in the ADL neurons. Ultimately, these findings will help us understand the mechanism by which neuropeptides modulate pheromone avoidance at the molecular and neural circuit levels.

Keywords: neuropeptide, ascarosides

727B The molecular mechanisms of light adaptation in the nematode *Pristionchus pacificus*

Aya Manabe[1], Keimei Ko[1], Kenichi Nakayama[2], Takahiro Chihara[1], Misako Okumura[11]Graduate School of Integrated Sciences for Life, Hiroshima University, [2]Department of Cell Biology and Center for Cell Dynamics, Johns Hopkins University School of Medicine

Most organisms can sense and adapt to a wide range of light intensities. Although animals commonly use opsins for light detection, the nematode *Pristionchus pacificus* lacks conserved photoreceptors such as opsins and LITE-1. We previously performed forward genetic screening using light avoidance in *P. pacificus* and found that the cyclic GMP signaling pathway and G protein-coupled receptor kinase are essential for light-avoidance behavior in *P. pacificus*. Although the mechanism of light sensing in *P. pacificus* has been partially elucidated, it remains unclear whether, and how, *P. pacificus* adapts to light. Here, we found that prior exposure to light reduced the frequency of light-avoidance behavior in *P. pacificus*, indicating its ability to adapt to light. To reveal the mechanism of light adaptation in *P. pacificus*, we used CRISPR/Cas9 genome editing to generate mutants in G protein β and γ subunits and arrestin, as they are involved in chemosensory adaptation in the nematode *Caenorhabditis elegans*. Gβ and Gγ subunit mutants exhibited light-avoidance behavior similar to that of the wild type, but light adaptation was impaired in the Gβ mutants. Similarly, the Gγ and arrestin mutants showed minor abnormalities in light adaptation. These findings suggest that these proteins play a role in sensory adaptation beyond that in chemosensation and could contribute to light response mechanisms in nematodes.

728B Repurposing the mitotic chromosome-microtubule coupling machinery to regulate axon termination

Vasileios Rafail Ouzounidis[1], Henrique Rafail Alves Domigos[2], Fernanda C Soberanis[2], Dhanya Cheerambathur[21]Institute of Cell Biology, University of Edinburgh, [2]University of Edinburgh

Precise neuronal patterning and connectivity relies on the ability of axons to form synapses with their correct partner cells. Upon reaching their target, axons terminate their growth and initiate synaptogenesis, a process that involves destabilization of the growth cones. While there is a growing understanding of the molecular mechanisms that promote and stabilize growth cone cytoskeleton during axon growth, the mechanisms responsible for destabilizing the growth cone cytoskeleton during axon termination remains unclear. Understanding and characterizing axon termination will bring us one step closer to elucidating the molecular mechanisms governing synaptogenesis. Here, we demonstrate that the kinetochore proteins, traditionally known for coupling microtubules to centromeric chromatin, to ensure proper chromosome segregation during mitosis, are repurposed in axons. We saw that these proteins promote proper axon termination of touch receptor neurons in *C. elegans*. Using fluorescence and electron microscopy we show that KNL-1, the central signalling and microtubule-binding scaffold of the kinetochore is enriched in the growing axons, and associates with membrane structures. Depletion of KNL-1 and its microtubule-binding partner NDC-80 resulted in enlarged growth cones, increased F-actin, and overextension of axons. Thus our findings have uncovered the chromosome segregation machinery as a key regulator of the axonal growth cone dynamics and proper axon extension. We are currently employing biochemical approaches to identify neuron-specific interactors, to investigate how the "neuronal kinetochore" promotes axon termination and synaptogenesis.

729B SRO-1, a homologue of mammalian Melanopsin OPN4, Modulates Light Avoidance Behavior in *Caenorhabditis elegans*

Kazuki Ozawa[1], Min Soo Shin[1], Yoichi Shinkai[2], Motomichi Doi[1,31]School of Life and Environmental Science, University of Tsukuba, [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), [3]Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)

Mammals possess intrinsically photosensitive retinal ganglion cells (ipRGCs) as a third class of photoreceptor cells, in addition to rods and cones (Dacey et al., Nature, 2005). Recent studies have shown that melanopsin (OPN4), expressed in ipRGCs, regulates circadian rhythms and mood in mice through blue-light reception (Tsai et al., PLOS Biol, 2009; Fernandez et al., Cell, 2018). In *Caenorhabditis elegans*, the gustatory receptor homolog LITE-1 is shown to function as a sole photoreceptive molecule and to detect short-wavelength light from blue to ultraviolet (Edwards et al., PLOS Biol, 2008). In addition, *C. elegans* respond to H2O2 generated by light exposure, along with GUR-3 and PRDX-2 (Quintin et al., PLOS ONE, 2022; Bhatla et al., Neuron, 2015). While *C. elegans* retains SRO-1, a homolog of melanopsin (OPN4), SRO-1 lacks the lysine residue essential for Schiff base formation with retinal (Troemel et al., Cell, 1995). It may not directly absorb photons and the function for blue-light reception remains unclear. In this study, we demonstrate that SRO-1 is involved in photoreception and regulates light avoidance behavior.

Phototaxis assays (Ozawa et al., Neurosci Lett, 2022) revealed that *sro-1* mutants exhibited reduced avoidance to UV (350 nm) and violet light (380 nm) compared to wild-type animals (N2). In a light avoidance assay by Ward et. al. (Nat Neurosci, 2008), *sro-1* mutants displayed normal avoidance under 488 nm illumination but showed a significant reduction in avoidance under 350 nm light compared to wild-type animals. Genetic analysis indicated that *sro-1* functions in the same genetic pathway with *lite-1*, but not with *prdx-2*, in regulating light avoidance behavior.

Ectopically expressed LITE-1 in muscle cells can induce forced egg ejection by the result of muscle contraction in response to blue light (Edwards et al., PLOS Biol, 2008). To know the role of SRO-1 in photoreception, we expressed SRO-1 in muscle cells ectopically and examined the response to light. We found that co-expression of SRO-1 and LITE-1 in muscle cells enhanced egg-laying at lower light intensities compared to LITE-1 alone, suggesting that SRO-1 may enhance photoreceptive response of LITE-1. Furthermore, expression analysis confirmed SRO-1 localization to the cell membranes of multiple neurons including PHA neurons where LITE-1 functions for light avoidance behavior. In conclusions, this study provides evidence that SRO-1 may contribute to *C. elegans* photoreception for light avoidance behavior through an uncharacterized photoreceptive mechanism.

730B The Characterization of a Novel Presynaptic Protein ROGDI in *C. elegans* Neurons

Beyza Ozen, Vasileios R. Ouzounidis, Dhanya Cheerambathur Institute of Cell Biology, The University of Edinburgh

Rogdi gene in humans is associated with Kohlschutter-Tonz syndrome, a rare autosomal recessive disorder characterized by a triad of epilepsy, amelogenesis imperfecta, and severe developmental delay. Although ROGDI is recognized as a potential presynaptic protein in mammalian systems, its precise molecular function remains unknown. Our work has identified H14A12.3, the orthologue of the Rogdi gene in *C. elegans*, as a neuron-specific interactor of kinetochore proteins in post-mitotic neurons. Typically known for their role in chromosome segregation, kinetochore proteins have recently emerged as critical regulators in the nervous system. In non-dividing neurons, they function as cytoskeletal regulators in dendrites and axons, contributing to the development and regeneration of neural circuits. In our GFP TRAP pulldown with the kinetochore protein KNL-1 from *C. elegans* primary neuronal cultures, H14A12.3 was one of the most highly enriched proteins. H14A12.3 shares a high degree of conservation with human ROGDI and exhibits a similar protein fold. Based on this structure, it has been suggested that ROGDI could serve as a platform for protein-protein interactions, despite the absence of known similarity to other protein domains. The function and expression of H14A12.3 have not been previously characterized in *C. elegans*. To address this, we generated a deletion strain and an endogenous GFP fusion to H14A12.3. Localization studies revealed that H14A12.3 is primarily expressed in neuronal tissues and becomes enriched in the nerve ring during late larval and adult stages. The deletion of the H14A12.3 appears to be superficially viable, and we are currently analyzing this strain for potential neuronal morphological or synaptic structural defects. Furthermore, we are conducting colocalization experiments with presynaptic proteins in neurons to gain more insights into the function of H14A12.3. Overall, these experiments in *C. elegans* neurons will provide insights into the function of a highly conserved and uncharacterized human ROGDI.

731B Identification of the Novel Ben Domain Containing Ben-a as A New Heterochronic Gene That Affects the Timing of Neuronal differentiation

Jill Pan Biological Science, University of Illinois Chicago

The transcription factor LIN-14 has recently been reported to contain a BEN domain. Reduction of function *lin-14* mutants display premature dendrite arborization and premature acquisition of neuronal maturation state based on neuronal maturation marker expression compared to wild type animals, indicating precocious neuronal differentiation. We are investigating if other BEN domain containing genes play a similar role in neurodevelopment. Using the PVD nociceptive neuron as a model system we determine whether the BEN domain containing *ben-a* (F53A2.3), like *lin-14*, affects the timing of neuronal differentiation by acting as a heterochronic gene. We found that *ben-a* mutants displayed precocious dendrite arborization and are in the process of using PVD neuronal maturation marker to verify precocious neuronal differentiation in *ben-a* mutants.

732B Exploring neuronal dynamics and therapeutic strategies in *C. elegans* models of *GNAO1* encephalopathy

Luca Pannone[1], Martina Di Rocco[1], Enrico Lanza[2,3], Serena Galosi[4], Manuela D'Alessandro[5], Valeria Lucente[2,3], Francesca C. Follo[1], Maria Novelli[4], Corrado Mazzaglia[2,3], Jean-Louis Bessereau[5], Elia Di Schiavi[6], Viola Folli[2,3], Vincenzo Leuzzi[4], Simone Martinelli[11]Oncology and Molecular Medicine, Istituto Superiore di Sanità, [2]Center for Life Nano Science, Istituto Italiano di Tecnologia, [3]D-tails, [4]Human Neuroscience, Sapienza University of Rome, [5]Univ. Lyon, University Claude Bernard Lyon 1, [6]Institute of Biosciences and BioResources, National Research Council

GNAO1 variants cause a group of childhood-onset neurological disorders characterized by developmental delay, drug-resistant seizures, and movement disorders. *GNAO1* encodes the α-subunit of an inhibitory G-protein (Gαo) regulating ion channel activity, neurotransmitter (NT) release, and neurodevelopment. Effective treatments are lacking, highlighting the need for a deeper understanding of disease mechanisms. Our recent work established *C. elegans* as a model for *GNAO1* encephalopathy. Genetically modified worms carrying pathogenic variants in *goa-1*, orthologue of *GNAO1*, behave as null mutants, exhibiting increased egg laying and hypersensitivity to aldicarb-induced paralysis. These findings suggest excessive NT release by different classes of neurons. *goa-1* mutants also show hyperactive and uncoordinated locomotion, which is rescued by caffeine through adenosine receptor antagonism.

Here, we explored the role of *goa-1* in individual classes of neurons and its connection to the observed phenotypes through *goa-1*(*RNAi*) and auxin-inducible degradation (AID) experiments. RNAi data identified cholinergic and GABAergic neurons as the primary drivers of hyperactive locomotion, while glutamatergic and dopaminergic neurons play a minor yet significant role, and revealed an unforeseen role for Gαo in GABAergic neurons. As a complementary approach, an AID-based system was developed and validated for conditional GOA-1 depletion in muscle or neurons. We then introduced a subset of *goa-1* variants into a strain expressing a pan-neuronal genetically encoded calcium indicator and recorded head ganglia neuronal dynamics using a custom-made microfluidic device. In *C. elegans*, two distinct neuronal modules control forward and backward movements; while activity within each module is highly correlated, activity between opposite modules is anti-correlated. *goa-1* mutants exhibited a frank dystonic phenotype characterized by reduced correlations and neurons spending most of their time transitioning between active and inactive states. Notably, each variant displayed a unique pattern of defects, mirroring the distinct phenotypic readout. Finally, we documented caffeine's ability to restore neuronal dynamics, highlighting its potential role in controlling *GNAO1*-related dyskinesia. Overall, the generated models provide new insights into disease mechanisms and establish an *in vivo* platform for studying dystonia.

733B Neuron-glia interactions through SAX-7/L1CAM maintain neuronal architecture

Marin Pascal[1,2], Marie Biard[1,2], Noémie Frébault[1,2], Ivan Valette[1,2], Zachary Grenier-Gosselin[1,2], Lise Rivollet[1,2], Claire Bénard[1,2,31]Department of Biological Sciences, Université du Québec à Montréal, [2]CERMO-FC Research Center, [3]University of Massachusetts Chan Medical School

The embryonically established nervous system needs to persist lifelong in the face of the animal's growth, maturation processes, body movements, and aging. While remarkable advances have uncovered mechanisms that drive nervous system assembly, the processes responsible for the maintenance of nervous system architecture throughout life is less well understood. One key protein that mediates long-term neuronal maintenance is the cell adhesion molecule SAX-7, which is homologous to the L1 protein family in vertebrates. In adult mice, L1CAM functions to safeguard cognitive abilities. In *C. elegans*, neuronal SAX-7 ensures that embryonically developed neuronal structures later maintain their proper organization and/or position, including of ganglia, fascicles, and the nerve ring. Interestingly, the gene *sax-7* is expressed not only by neurons but also by glial cells, including glia surrounding the nerve ring, but the role in these glia remains to be addressed. Here, we study the role of nerve-ring associated glial cells CEPsh and GLR in neuronal maintenance, and the contribution of SAX-7 in this process. For this, we generated tools to visualize glia and neurons simultaneously and examined *sax-7* null mutants. We show that SAX-7 loss profoundly affects the morphology and position of CEPsh and GLR glial cells, and that these *sax-7* glial phenotypes are correlated with -and precede- neuronal defects. To elucidate the role of SAX-7 in this mechanism, we are conducting rescue assays of neuronal and glial defects by restoring *sax-7(+)* specifically in neurons and/or glia, as well as tissue-specific depletion of SAX-7 by AID. Furthermore, we previously showed that the protein SAX-7 is cleaved and that the resulting fragments together ensure function in neuronal maintenance. We are thus testing the functional requirement for the SAX-7 cleavage in preserving both neuronal and glial morphology/position by using precise targeting of the SAX-7 cleavage site in the endogenous locus. By studying the impact of glia in neuronal maintenance and the conserved protein SAX-7/L1CAM, our work is expected to shed light on nervous system maintenance mechanisms and help decipher processes that go awry in neurodegenerative conditions.

734B PXF-1 promotes presynaptic assembly through multiple small GTPases

Reagan Lamb, Kate Pauss, Salvatore Cherra Neuroscience, University of Kentucky College of Medicine

Small GTPases are key regulators of synaptic regulation and neuronal development the activity of small GTPases is modulated by pairs of Guanine Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). Mutations that offset the careful equilibrium of these pathways are associated with intellectual disability, epilepsy, and other neurodevelopmental disorders. The *Caenorhabditis elegans* RapGEF homolog PXF-1 is involved in cholinergic neuron development, but it is uncertain which GAP proteins are a part of this pathway. Therefore, we sought to identify the GAPs associated with the PXF-1 pathway. Since Rap and Ras GTPases are part of the same subfamily of small GTPases, we investigated whether RasGAP proteins GAP-1 and GAP-2 influenced synapse development in the presence and absence of a pxf-1 mutation. We measured the intensity of cholinergic synaptic vesicle markers and found that *gap-1* mutations restored the intensity of synaptic vesicle markers in *pxf-1* mutants. Since the canonical target of GAP-1 is LET-60, we investigated whether LET-60 functions downstream of pxf-1. We expressed a constitutively active version of LET-60 in neurons and measured the intensity of the synaptic vesicle marker mCherry::RAB-3 expressed in cholinergic motor neurons. We observed a significant increase in the intensity of mCherry::RAB-3 labeled vesicles in pxf-1 mutants expressing a constitutively active LET-60. Together these data indicate that PXF-1 influences the activity of LET-60, which is antagonized by GAP-1. These data suggest that PXF-1 activity begins a cascade of GTPases regulating synaptic development in cholinergic neurons, including the Ras and Rap GTPases. In future studies, we will investigate the third putative RasGAP GAP-3 for potential interaction with the PXF-1 pathway. Further investigation of the PXF-1 pathway may elucidate a precise order of operations for neuronal development.

735B bHLH-mediated regulation of Neuronal CUT Homeobox gene expression

Karinna M Pe, Oliver Hobert Biological Sciences, Columbia University

How are broadly shared neuronal characteristics regulated in the *C. elegans* nervous system? A family of six CUT homeobox genes jointly regulate a battery of pan-neuronal factors, including proteins involved in synaptic vesicle cycling and neuropeptide processing, in a dose-dependent manner. Of these CUT genes, only *ceh-44* and *ceh-48* are localized to the nervous system. While *ceh-44* appears to be regulated via UNC-75-mediated alternative splicing, the mechanisms underlying the nervous system specificity of *ceh-48* remain unknown. Proneural basic helix-loop-helix (bHLH) transcription factors are conserved drivers of neuronal fate via heterodimerization to a partner protein and subsequent binding to canonical E-box binding motifs. Through promoter bashing, we identified E-box containing enhancers in the *ceh-48* upstream regions. Using a GFP-tagged *ceh-48* reporter strain, we have found that the mutation of E-box cis-regulatory motifs leads to an almost but not entirely complete elimination of *ceh-48* gene expression. Similarly, crossing the *ceh-48* reporter allele into various bHLH mutant lines significantly reduces *ceh-48* pan-neuronal expression on a nervous system-wide scale. The extent to which *ceh-48* expression is reduced in the E-box mutants is approximately equivalent to that seen in the bHLH mutant crosses. Because these mutations do not result in a complete loss of *ceh-48* expression, it is likely that non-bHLH factors are jointly involved in its regulation. Taken together, our findings shed light on the underlying mechanisms of pan-neuronal identity regulation in the *C. elegans* nervous system.

736B Physics lessons from worms: How a strange worm behavior revealed electromagnetic sensing of water flow

Laura Persson[1], Noelle L'Etoile[21]Cell and Tissue Biology, University of California, San Francisco, [2]UCSF

Sensory systems evolved to detect physical cues that provide relevant information about the environment. In this way, they offer a unique window into both biology and physics. Indeed, efforts to understand our own visual system led to fundamental breakthroughs in electromagnetism. C. elegans evolved in a very different physical environment, one in which moist organic matter is the more relevant medium than air, and their sensory systems should reflect this. We discovered that C. elegans has its own electromagnetic sensing system tuned, not to the wavelengths that we see as visible light, but to a set of electromagnetic signals arising from the flow of salt water. C. elegans respond to minute flows (~ums/hour) induced in their agar substrate through temperature-driven convection, ion concentration gradients, surfactants, or physical wicking. Animals migrate to the source of ion outflow, and stable flows evoke striking collective patterning. The extremely slow nature of the flow made it unlikely that animals could be responding directly to shear stress. Moreover, we found that their capacity to sense flow extends to solutions with which they have no physical contact at all. Indeed, animals within a sealed petri dish will robustly localize toward a solution in a nearby sealed petri dish provided it has ions and a source of flow. To make sense of these perplexing observations, we took a "principles first" approach. We reasoned that a signal arising from the movement of electric charges and sensed remotely could be electromagnetic in nature. In the simplest case, we can imagine a set of fixed negative charges (the acidic side chains of the agar) and a set of mobile positive charges (protons). In this system, ordered flow would create the inverse of a typical electrical wire (mobile negatives with fixed positives), and the predicted signal is a magnetic field (if we consider charge pairs in which both are moving we get more complex EM signals). Accordingly, we can measure a magnetic field in the nTesla range due to flow in the agar and find that this field magnetizes iron particles. A Faraday Cage impedes localization to an adjacent solution and magnetic shielding fully inhibits it. Mutants defective in the AFD neuron (proposed to be involved in sensing Earth's magnetic field) are defective in flow sensing. This work challenges basic assumptions about sensory biology and reveals a likely ubiquitous signal providing a rich source of environmental information.

737B G-Protein Receptor Kinase 2 is Necessary for Sickness Sleep in *C. elegans*

David M Raizen, Marisa C Petticord Neuroscience, University of Pennsylvania

Sleep during sickness (SIS) is widely observed across phylogeny, but its mechanism is poorly understood. We (William J. Lee et al) recently identified 42 SIS-inhibiting chemicals, which included the anti-depressant mirtazapine. To understand how mirtazapine inhibits SIS, we are taking an unbiased forward genetic approach that consists of screening million-mutation project (MMP) strains for resistance to the wake promoting effects of mirtazapine. We use UV radiation to induce sickness sleep. We identified an MMP strain with resistance to the wake-promoting effects of mirtazapine but normal response to other wake-promoting chemicals. The selective resistance to mirtazapine suggests that the resistance is the result of pharmacodynamics rather than global pharmacokinetic differences. This MMP strain harbors a null mutation in the gene *grk-2*, which encodes a G-protein coupled receptor kinase. An independent *grk-2* knockout strain we obtained from the CGC had a similar phenotype to the MMP strain and the two *grk-2* mutants failed to complement each other, demonstrating genotype-to-phenotype causality. We are now testing potential hypotheses by which mirtazapine and GRK-2 regulate sickness sleep.

738B Inter-class axon-axon interaction defines tiled synaptic innervation of DA-class motor neurons in *C. elegans*

Federico A Pini, Mizuki Kurashina, Annie Ou, Kota Mizumoto University of British Columbia

Axon-axon interaction plays a crucial role in topographic map formation during nervous system development. However, we have limited knowledge about how it regulates neuronal map formation at the level of synapse patterning. Previously we showed that Semaphorin-Plexin signaling mediates the inter-axonal interaction between two DA-class cholinergic motor neurons, DA8 and DA9, to define their tiled synaptic innervation (Mizumoto and Shen PMID: 23439119). As the axons of DA8 and DA9 have contact with other axons within the dorsal nerve cord, including those of DB7 and DD6, we tested the potential contributions of other neuron classes in the synaptic tiling of DA neurons. We found that genetic ablation of the DB class of cholinergic motor neurons using the recCaspase-3 system resulted in a moderate but significant synaptic tiling defect between DA8 and DA9. Consistently, DA8 and DA9 synaptic tiling is also disrupted in the *vab-7(e1562)* mutant, in which the axons of DB neurons, including DB7, are often misguided anteriorly. On the other hand, genetic ablation of D-type GABAergic motor neurons (DDs and VDs) did not cause a synaptic tiling defect of DA8 and DA9, suggesting that the DB axons, particularly the DB7 axon which overlaps with the DA8 and DA9 synaptic regions, are specifically required for the synaptic tiling of DA8 and DA9. We conducted a candidate screening to identify cell adhesion and signaling molecules mediating DA-DB interaction and found that a null mutant of *syg-2(miz185)*, which has a nonsense mutation in exon 12, exhibited a moderate synaptic tiling defect between DA8 and DA9 similar to *vab-7(e1562)* mutants. We found that *syg-2(miz185)* did not enhance the synaptic tiling defect resulting from DB ablation (recCaspase-3 or *vab-7*) suggesting that *syg-2* may act through DB7 to control DA8-DA9 synaptic tiling. Interestingly, *syg-2(miz330)* mutants lacking the cytoplasmic domain also displayed synaptic tiling defects between DA8 and DA9, suggesting that SYG-2 may signal through its cytoplasmic domain to control DA8-DA9 synaptic tiling. We also found that a null mutant of *syg-1(ok3640)*, a known binding partner of *syg-2*, also displayed a tiling defect between DA8 and DA9; however, it is less severe than that of *syg-2(miz185)* mutants. Unlike SYG-2, the cytoplasmic domain of SYG-1 is not required to regulate synaptic tiling between DA8 and DA9. Currently, we are continuing to investigate the role of *syg-2* and *syg-1* and the tissues in which they function.

739C Targetting Charcot-Marie-Tooth (CMT) disease in the nematode *Caenorhabditis elegans*

Emili Plastira, Maria Dimitriadi Clinical, Pharmaceutical and Biological Science, University of Hertfordshire

Charcot-Marie-Tooth (CMT) disease, one of the most common inherited heterogeneous disorders, has been reported to affect 2.5 million people worldwide. It mainly causes neurological defects with a primary target being the peripheral nervous system. It also contributes to muscle weakness and length-dependent degeneration of motor and sensory axons. However, it has not been determined the genotypic and phenotypic factors associated with CMT and how the disease progresses. Recently, identification of biallelic loss-of-function mutations in the *SORD* gene are found to be related with the most prevalent CMT recessive form. Crucial is the role of CMT-*SORD* in the second part of the polyol pathway, where *SORD* contributes to the interconversion of glucose into fructose via sorbitol. Mutations in the *SORD* gene can cause alterations in the polyol pathway, leading to significantly higher levels of sorbitol in patients' serum and fibroblasts. Hence, peripheral nerves will further face toxic effects. More specifically, elevation of the sorbitol levels will result in osmotic stress, ROS production, and mitochondrial dysfunction. Interestingly, the increased of sorbitol in plasma constitutes a crucial biomarker for the disease as sorbitol levels have been associated with disease diagnosis and the development of treatment strategies. Driven by the need for reducing CMT defects, we delve into CMT by mimicking human *SORD* gene mutations in the nematode *C. elegans*. By utilizing *C. elegans* as promising platform for current investigation, we aim to uncover neuromuscular defects and behavioral changes linked with *SORD* mutations to further elucidate the cellular and molecular mechanisms underlying CMT pathology.

740C EGL-4 wears many hats to turn sensory information into proteostatic action

Tatiana Popovitchenko[1], Eunchan Park[1], Julia Galesi[1], Yeshaswi Pulijala[1], Mehul Vora[2], Christopher Rongo[1]1Genetics, Rutgers University, [2]2ModOmics Ltd

The foraging gene in *C. elegans*, *egl-4*, integrates external gaseous milieu with internal sensory states. This complicated function is also in the context of diverse pleiotropic phenotypes in *egl-4* loss-of-function mutants, including the eponymous egg-laying defect, impaired olfaction, and a newly discovered phenotype of impaired escape behavior under hypoxia. Yet, molecular understanding of the role of EGL-4 in most of these diverse pathways is unknown. Here, we tested whether EGL-4 might regulate hypoxia-induced escape behavior through regulation of GLR-1, an AMPA-type glutamate receptor that acts in the command interneuron circuit to regulate the forward-reverse decision for movement. We found that loss-of-function mutations in *egl-4* result in a GLR-1 subcellular distribution pattern and roaming behavior that resembles hypoxia-treated animals. Using CRISPR-Cas9 editing, we next introduced mNeonGreen (mNG) and an Auxin Inducible Degron (AID) into the endogenous *egl-4* locus. Crossing this allele into the NeuroPAL worm revealed that EGL-4 is co-expressed with GLR-1 in the command interneurons. We generated a transgene that expresses TIR1, the auxin-activated ubiquitin ligase that recognizes the AID tag, in the command interneurons to test the cell autonomous requirement for EGL-4. We next explored the broader molecular pathways that EGL-4 commands to enact its many roles. A major unknown has been direct transcriptional targets, which we uncover with an RNAseq study of both loss- and gain-of-function mutants. Finally, we uncovered post-transcriptional changes in fluorescent-based reporters in *egl-4* mutants, including reporters sensitive to changes in the Ubiquitin Proteasome System (UPS). Our results suggest that the pleotropic effects of *egl-4* mutations might be due to impaired protein turnover, and that EGL-4 might function to integrate sensory information and then direct appropriate systemic changes in proteostasis and stress response.

741C Regulation of glutamatergic synapses and behavior by neurexin and calsyntenin

Regina Powers[1], Camille Hu[2], Peter Juo[21]DMCB, Tufts University School of Medicine, [2]Tufts University School of Medicine

Proper development and function of glutamatergic synapses is critical for brain function, and dysregulation of glutamatergic synapses leads to neurodevelopmental and neuropsychiatric disorders such as Autism Spectrum Disorder (ASD) and Schizophrenia. Neurexin-1 is an adhesion molecule implicated in neurodevelopmental disorders that regulates development. However, the mechanisms by which neurexin mediates glutamatergic synapse development, particularly *in vivo*, are not completely understood. Investigating the function of neurexin *in vivo* in mammals has been challenging as there are 3 neurexin genes that encode two major isoforms each, along with thousands of alternatively spliced isoforms that are differentially expressed throughout the brain. In contrast, *C. elegans* has a single neurexin homolog, *nrx-1*, that has 2 major isoforms and a handful of predicted alternatively spliced isoforms. Here, we used *C. elegans* to investigate the role of *nrx-1* on glutamatergic synapse development and function. In contrast to a previous study, which found no effect of *nrx-1* mutants on glutamatergic synapses labeled by overexpressed GLR-1::GFP, we found that *nrx-1* mutant animals have decreased levels of GLR-1 at synapses using endogenously labeled GLR-1-GFP. In addition, we found that GLR-1 levels decreased at synapses in the ventral nerve cord in a region-specific manner. This decrease is dependent on the long *nrx-1-alpha* isoform. *nrx-1* mutant animals also show deficits in ASH-mediated behavior and spontaneous locomotion reversals, both of which are mediated by glutamate and GLR-1. We found that loss of *nlg-1* (neuroligin), the most well-studied trans-synaptic binding partner of neurexin, did not affect GLR-1 levels. Interestingly, we found that loss of *casy-1* (calsyntenin), a less well-characterized binding partner of neurexin, also results in decreased GLR-1 at synapses. Genetic double mutant analysis of *nrx-1* and *casy-1* suggests that these genes function together in the same genetic pathway. We are currently investigating the effects of *nrx-1* and *casy-1* mutants on the pre-synaptic compartment, and conducting rescue experiments to determine if *nrx-1* and *casy-1* are functioning pre- or post-synaptically. We are also investigating the effect of a conserved ASD patient mutation in *nrx-1* on glutamatergic synapses. This study will uncover molecular mechanisms by which neurexin mediates synapse development *in vivo*.

742C NLP-67 and its receptor NPR-28 regulate stress-induced sleep in *C. elegans*

Vishnu Raj[1], Luca Golinelli[2], Isabel Beets[3], Han Wang[11]University of Wisconsin, [2]KU Leuven, [3]Animal Physiology and Neurobiology, KU Leuven

Sleep is a highly conserved physiological process in the animal kingdom. In *C. elegans*, cellular stress triggered by external stressors such as heat, UV, and pathogens can cause stress-induced sleep (SIS), which is characterized by cessation of feeding and body movement, as well as reduced responsiveness to the environment. SIS is primarily mediated by the activation of epidermal growth factor receptor (EGFR) in the ALA neuron. However, the downstream molecular pathways regulate different behavioural features during SIS are still not fully understood. Through an unbiased forward genetic screen, we identified *nlp-67*, a neuropeptide like protein gene, as a novel sleep regulator for SIS. We found that *nlp-67* is expressed in the ALA neuron. *nlp-67* mutants were defective in feeding quiescence, while overexpression of *nlp-67* specifically induced feeding quiescence. We identified NPR-28, an allostatin C type GPCR, as the receptor for NLP-67. NLP-67-induced feeding quiescence can be suppressed by null mutations of *npr-28*. The mature NLP-67 peptide can activate NPR-28 in a heterogeneous expression system. A transcriptional reporter using a cGAL gene trap allele of *npr-28* showed that it is strongly expressed in head neurons, pharyngeal muscles, and intestine cells. Our preliminary results suggest that NPR-28 functions in neurons to control feeding quiescence. Together, our data show the allostatin signalling through NLP-67 and its receptor NPR-28 specifically regulates feeding quiescence during SIS. This novel neuropeptide-GPCR interaction and the underlying molecular mechanisms will advance our understanding of neuronal basis of sleep.

Key words: EGFR; Stress-Induced Sleep (SIS); NLP-67; NPR-28

743C Ingestion of A53T mutant alpha-synuclein expressing *E. Coli* induces behavioral deficits in *C. elegans*

Srikrishnan Raju[1], Greg J. Phillips[2], Danielle Mor[11]Medical College of Georgia, [2]University of Georgia

Abnormal aggregation of alpha-synuclein protein has been found to trigger progressive dopaminergic neuronal degeneration in Parkinson's Disease (PD), yet the mechanisms underlying alpha-synuclein toxicity remain incompletely understood. Growing evidence points towards a gut-to-brain, prion-like propagation of alpha-synuclein misfolding. Studying the mechanisms of neurodegeneration in PD is challenging due to the long aging process and low genetic tractability of rodent models. However, the rapid development and aging of *C. elegans* and high tractability make them uniquely suited for age-related neurodegenerative disease research. Our lab previously created a gut-to-brain alpha-synuclein PD model by feeding *C. elegans* pre-formed fibrils (PFFs) of human alpha-synuclein, inducing dopaminergic neuronal degeneration and accompanying behavioral deficits. A potential limitation of this model is the possibility that PFFs are absorbed through the epithelium rather than strictly ingested by mouth. Here, we show a complementary *C. elegans* model of gut-to-brain alpha-synuclein transmission, in which worms are fed *E. coli* expressing human alpha-synuclein with the PD-linked A53T mutation. Using *E. coli* expressing mutant alpha-synuclein ensures the alpha-synuclein is exclusively ingested orally. We have found that feeding worms this source of alpha-synuclein produces age-dependent dopaminergic behavioral deficits. In addition, our preliminary data suggest that this functional deficit precedes any structural defects in dopaminergic neurons. This work offers a new model that can be used as a high-throughput platform for investigating the age-dependent role of the gut-to-brain axis in PD progression and identifying therapeutic interventions.

744C MACSPI enables tissue-selective proteomic and interactomic analyses in multicellular organisms

Qiao Ran[1], Siyue Huang[1], Xiaomeng Li[2], Xiucong Bao[1], Chaogu Zheng[1], Xiang David Li[21]School of Biological Sciences, The University of Hong Kong, [2]Department of Chemistry, The University of Hong Kong

Although advances in RNA-sequencing technologies enabled transcriptomic profiling of specific cell types, it remains challenging to profile the proteomes of specific cells in intact tissues or animals. Understanding gene expression at the protein level in the nervous system is particularly difficult given the diversity of neuron types and the fact that the physical isolation of neurons often leads to the loss of proteins in dendrites and axons. Moreover, proteins work in complexes, and currently, there is a lack of robust methods to study protein-protein interaction in specific cells.

To address the technology gap, we developed Methionine Analog-based Cell-Specific Proteomics and Interactomics (MACSPI) methods to profile the proteomes and interactomes of cells of interest in intact *C. elegans*. We first synthesized an unnatural amino acid that is a methionine analog containing both a bio-orthogonal tag and a photo-crosslinker; we then engineered a methionyl-tRNA synthetase (MetRS) mutant that can charge the unnatural amino acid onto tRNA(fMet) and expressed the MetRS mutant into specific tissues, including the body wall muscles, all neurons, and specific neurons. Through click-chemistry, protein enrichment, and liquid chromatography-tandem mass spectrometry (LC-MS/MS), we showed that the chemical probe can label the proteins from specific cells in the whole worm lysate and allow the proteomic profiling of these cells. Combining this method with a modified SILAC approach, we profiled muscle and neuronal proteomes and identified novel tissue-specific proteins. Furthermore, we showed that this method can also be used to profile and compare the proteomes of specific neuron types by profiling the proteomes of the touch receptor neurons and the dopaminergic neurons.

For tissue-specific interactomics, we used the chaperone HSP-90 as an example and first create a strain that tags the endogenous HSP-90 with a HA tag. We then used UV-induced photo-crosslinking and double-enrichment with both the bio-orthogonal tag and HA tag to profile muscle and neuron-specific HSP-90 interactors and demonstrated the profiling of chaperone interactomes in specific tissues. We further profiled and compared the muscle and neuronal HSP-90 interactomes under physiological and thermal stress conditions and identified tissue-specific and stress-responsive HSP-90 interactors. We are in the process of extending this method into other chaperone proteins in attempts to create a tissue-specific chaperone interactome map.

745C Caenorhabditis arcana

James Rand Oklahoma Center for Neuroscience

During many years of studying cholinergic function, we sometimes encountered experimental data that were unexpected, perplexing, intriguing, and worthy of further study. Unfortunately, the converging pressures of limited time, limited funding, and unlimited entropy meant that in some of these cases, the further study never happened, and even after 25, 35, or even 45 years, some of these issues remain puzzling. Here are a few examples:

Humoral acetylcholine: extrasynaptic transmitter diffusion and action at a distance, now called "volume transmission," has been documented in numerous organisms for transmitters such as GABA and dopamine. There is also evidence for humoral acetylcholine in several organisms, including *C. elegans*. However, the precise source of the acetylcholine is unclear. Reporter constructs driven by the *unc-17* promoter show moderate to strong expression in a group of midbody cells tentatively identified as the four uv1 cells. The uv1 cells would be an ideal source of humoral acetylcholine, because they are known to be secretory and they have unencumbered access to the pseudocoelom. Unfortunately, there is no uv1 staining with anti-UNC-17 or anti-CHA-1 antiserum.

Ghost Promoter: In the cholinergic (*unc-17 - cha-1*) locus, there is a cluster of 14 conserved sequence motifs in the intron between the 1st and 2nd *unc-17* coding exons. This cluster is conserved among the 22 members of the Elegans Group and the 13 members of the Japonica Group; it has the characteristics of a promoter, but has no apparent function. In some species, there is a genomic inversion, inverting the entire motif cluster in the intron. Cladograms of *Caenorhabditis* species have a branch of the Elegans Group consisting of 7 species: *C. brenneri, C. doughertyi, C. tropicalis, C. wallacei, C. sp44, C. sp48 and C. sp51,* and these 7 species, and only these 7 species, contain this genomic inversion.

Three extragenic suppressors of *unc-17(e245)* mutations have been identified: *sup-1, sup-2/erd-2*, and *sup-8/snb-1*. Based on these 3 suppressors, we developed a strategy for identifying additional suppressing genes. We also isolated several intragenic *e245* suppressors; these suppressors and the proteins they encode could be useful tools for study of the mechanism of UNC-17 transport.

746C Phenomic characterization of *C. elegans* orthologs of Parkinson's Disease-Associated genes leads to identification of best candidate for GWAS hit

Catharine Rankin, Joseph Liang, Kristen Tsoi, Anuska Sood, Christopher Mok, Ben Westmore Psychology, Univ British Columbia

Parkinson's Disease (PD) is the most common neurodegenerative movement disorder affecting up to 1% of people >65 years old. Our understanding of the genetic contributions of PD has been expanded by advances in genetic studies in recent years. However, two major challenges plague researchers studying the genetic underpinnings of the disease today: 1) There is a need to functionally characterize newly identified risk loci, and 2) a signification portion of GWAS loci do not have a best gene candidate assigned. We established a pipeline for *in vivo* characterization of *C. elegans* orthologs of newly identified PD risk loci. *C. elegans* have orthologs of many PD-associated and biologically relevant genes. Our lab developed the Multi-Worm Tracker for high-throughput characterization of behavioural and morphological phenotypes in populations of freely behaving animals in real time. Studying more than 150 mutant strains harbouring loss-of-function mutations in orthologs of PD-linked genes has yielded unique phenotypic profiles spanning up to 30 phenotypes ranging from morphology, baseline behaviours, non-associative learning and a dopamine-dependent behaviour for 83 PD-linked gene orthologs. With data from this, and from past screens for genes expressed in neurons, glia and involved in ASD, we identified a subset of phenotypes that are most reflective of the PD dataset. Based on this insight, we are addressing the second problem by building a machine learning model to propose the best candidate causal gene for risk loci that currently do not have a top candidate in the PD GWAS Loci Browser. Using this model, a preliminary screen of 6 candidate genes surrounding locus 71 identified TBX2·tbx-2 with a high degree of confidence. Ultimately, this research will establish high-throughput genotype-to-phenotype characterization of newly identified risk genes for PD and highlight new PD risk genes of high interest for future down-stream disease modelling efforts and further our understanding of the biological processes underlying PD.

747C New mechanisms of embryonic brain assembly; focus on a non-canonical Hedgehog-like pathway

Georgia Rapti[1,2,3,4,5], Francesca Caroti[1], Chiara Mungo[1], Lautaro Gandara[1,21]Developmental Biology Unit, Epigenetics and Neurobiology Unit, European Molecular Biology Laboratory, [2]Developmental Biology Unit, European Molecular Biology Laboratory, [3]Epigenetics & Neurobiology Unit, European Molecular Biology Laboratory, [4]FENS-Kavli Network of Excellence, [5]Interdisciplinary Center of Neurosciences, Heidelberg University

Tissue functionality relies on complex architecture emerging from cellular and molecular interactions. In neural networks, operational complexity reflects intricate circuit architecture formed by neurons and glial cells— the understudied non-neuronal cells. Axons and glial cells develop together, shaping intricate morphologies that define connectivity. Disrupting circuit architecture is linked to neurodevelopmental or neurodegenerative pathologies, emphasizing the need to understand circuit assembly *in vivo*. Despite extensive neurodevelopment studies, we lack a full mechanistic view of circuit morphogenesis in live embryos due to embryonic inaccessibility and mechanistic complexity. To address these, we leverage *C. elegans* powerful transparency, stereotyped anatomy, genetic manipulability, and single-cell imaging.

Our studies uncover that brain assembly *in vivo* is embryonic and hierarchical. We identified specific pioneer neurons and glia guiding follower cells via conserved pathways. These glia are analogous to vertebrate astroglia in architecture, fate, and function, suggesting our findings may have broad implications. We now dissect new mechanisms of pioneer neuron and glial formation (Sabou, Bhushan, Cibulskaite, Pal, Rapti, unpubl.). We also dissect the fidelity of assembly that relies on molecular synergies. We uncover that compensatory mechanisms mask synergistic implications. To identify hidden factors of assembly, we developed modifier screens combining sensitized backgrounds, gene manipulation, and imaging (Caroti, Mungo, Rapti, unpubl.). We found that embryonic brain formation relies on a non-canonical Hedgehog-like pathway, largely unexplored due to expansion and missing factors. Unlike its vertebrate homologs, this pathway is not linked to embryonic brain formation. We now implicate several pathway components in embryonic brain assembly and study their interactions *in vivo*, by leveraging a novel dominant mutant we generated. We also assess *in vivo* spatio-temporal roles of the implicated factors in glia/neuron morphogenesis, from embryogenesis up to the functional circuit outputs, by combining cross-scale studies from embryonic imaging to animal behavioral assays. We show that the non-canonical Hedgehog-like pathway mediates epithelia, astroglia, and neuron interactions driving embryonic brain development. Interestingly, this C. elegans Hedgehog-like pathway lacks Smoothened, a key factor for downstream signaling present in other organisms. We explore conserved factors that may compensate for this absence, offering insights into putative ancestral molecular signaling mechanisms ensuring circuit assembly fidelity *in vivo*.

748C Dopamine Receptor D2 contributes to the phenotypic severity in a *C. elegans* model of spinal muscular atrophy

Saman Rashid, Ariadna Garrido García, Lucía Pérez García, Coral Crespo Gómez, Maria Dimitriadi School of Health, Medicine and Life Sciences, University of Hertfordshire

Spinal muscular atrophy (SMA) is a devastating neuromuscular disorder characterised by the degeneration of lower α-motor neurons, resulting in progressive muscle loss and ultimately death. SMA is caused by reduced levels of the ubiquitously expressed survival motor neuron (SMN) protein, although it remains unknown why SMN depletion specifically affects motor neurons. Autophagy is a lysosomal-degradation pathway involved in the removal of protein aggregates and aged organelles; thus, the pathway is particularly important in neurons. Findings from SMA mammalian and cell culture studies have highlighted a reduction in autophagic activity that is characterised by reduced autophagic flux when SMN levels are depleted. We aimed to target the autophagic pathway as a potential therapeutic avenue in the well-established *Caenorhabditis elegans* (*C. elegans*) SMA model. Our transcriptomic analysis revealed an upregulation of autophagy genes, although this was coupled with reduced lysosomal biogenesis suggesting defects in degradation. Moreover, using a reporter strain for autophagic flux, we validated the findings of our transcriptomic analysis and found an accumulation of autolysosomes which were likely to be non-degradative. We then pharmacologically challenged the nematode by exposing animals to various modulators of autophagy. We found that fluphenazine, a D2 receptor antagonist and autophagy activator, was capable of ameliorating SMN defects by increasing (a) pharyngeal pumping, (b) locomotion, (c) survival and (d) endogenous SMN protein levels. Subsequently, we identified the D2 ortholog *dop-3* as the molecular target of fluphenazine, where *smn-1;dop-3* knockout animals exhibited a phenotype similar to that induced by fluphenazine treatment. Finally, when autophagy was inhibited in *smn-1;dop-3* knockout mutants, the partial rescue in phenotype was abolished. Our results highlight a dopamine-autophagy signaling axis as a key contributor to SMA defects and pinpoint *dop-3* as a genetic modifier of SMA in *C. elegans*.

749C OpenAutoScopeV2: A single-worm tracker to probe behavior and physiology throughout the lifetime of a worm

Sina Rasouli[1], Mahdi Torkashvand[1], Jeremy T. Florman[2], Jinmahn Kim[1], Maedeh Seyedolmohadesin[1], Sahana Gangadharan[3], Ilana Albert[1], Cassi Estrem[4], Catherine Marshburn[5], Steven W. Flavell[4], Mark J. Alkema[2], Vivek Venkatachalam[11]Physics, Northeastern University, [2]Neurobiology, UMass Chan Medical School, [3]Bioengineering, Northeastern University, [4]Picower Institute for Learning and Memory, Massachusetts Institute of Technology, [5]Behavioral Neuroscience, Northeastern University

Microscopes to track single worms have allowed, among other things, the study of decision making (Faumont, et. al 2011, Cermak, et. al 2020), phenotypic response characteristics (Yemini, et. al 2013), circuitry underlying foraging (Ben Arous, et. al 2009, Stern 2017), and the correlation of neuronal activity to behavior (Faumont, et. al 2006).

Here we integrated technologies from many labs to build a single worm tracker capable of the following: (1) We've improved tracking (XYZ) and worm housing to continuously image an animal as it progresses through all larval stages (up to 60h). (2) We've improved optics, added excitation and emission paths, and characterized acute and chronic blue light toxicity to allow fluorescent imaging of calcium dynamics or gene expression throughout development. (3) We've added the capability for optogenetic stimulation and mechanical stimulation via a solenoid tapper. (4) We've added the ability to perform calcium imaging in microfluidic devices as worms are exposed to different chemicals. (5) We've added software to allow long-running automated experiments that trigger interventions based on sensory or behavioral events (e.g. approaching a specific food patch or beginning to reverse).

We present several examples of this tool in use. (1) We captured single-neuron activity from the NSM neuron upon food entry throughout development. We additionally captured the cyclically varying expression of a transcription factor (GRH-1, reported via GFP) matching dynamics observed in confocal systems (Meeuse, et. al 2023). (2) We performed high magnification single neuron calcium imaging in microfluidic chips as animals respond to multiple odorants. (3) We conducted simultaneous behavioral and Ca2+ imaging of the tap-induced escape response. We observed increased RIM Ca2+ activity correlating with reversal and omega turns, as well as a delay in intestinal Ca2+ oscillations and defecation behavior in response to the tap stimulus. (4) We imaged and characterized behavioral responses to optogenetic stimulation in mechanosensory neurons across different larval stages.

To maximize the impact of this technology, we have made the microscope design, software, and step-by-step building tutorials freely available in an open-source repository. The microscope is compact and can be easily assembled within 2 days with readily available components at low cost.

750C Mitochondrial stress in the GABAergic neurons non-cell-autonomously regulates organismal health and longevity in *Caenorhabditis elegans*

Laxmi Rathor[1], Sung Min Han[21]Physiology and Aging, University of Florida, [2]2Department of Physiology and Aging, Institute on Aging, University of Florida

Mitochondrial stress is associated with diseases, health, and aging. Emerging studies suggest that mitochondrial stress in the nervous system can trigger non-cell-autonomous responses in peripheral tissues. However, the specific neurons involved and their systemic effects on the health and aging of the organism remain incompletely understood. Using *Caenorhabditis elegans* (*C. elegans*) as an *in vivo* model, we found that mitochondrial stress gamma-aminobutyric acid (GABA) neuron was sufficient to significantly alter organismal lifespan, stress tolerance, and reproductive capacity. This stress also increased mitochondrial mass in peripheral tissues, which in turn affected energy production and reactive oxygen species (ROS) levels. Notably, mitochondrial stress in GABAergic neurons enhanced DAF-16/FoxO activity, which was required for these non-cell-autonomous effects. The GABA signaling was necessary for the enhanced DAF-16/FoxO activity following mitochondrial stress in GABAergic neurons. Collectively, our data suggests that local mitochondrial dysfunction in GABAergic neurons could influence mitochondrial homeostasis, health, and aging of the organism through the DAF-16/FoxO pathway.

Keywords: *Caenorhabditis elegans,* Mitochondria, GABA neurons, Aging, Health

751C Neuropeptides regulate the plasticity and function of a mature, dimorphic neuron in *C. elegans*

Vina Tikiyani, Tammy Ray, Evan Li, Michael P Hart University of Pennsylvania

Neuronal remodeling and circuit plasticity are essential for learning and behavioral modulation. Our lab investigates experience-dependent neuronal remodeling in the GABAergic DVB neuron, characterized by progressive neurite outgrowth in adult males (which does not occur in hermaphrodites). The plasticity of DVB directly regulates spicule protraction, a key step in male mating behavior, serving as a functional readout of DVB neuronal remodeling. Our lab has identified numerous genes and molecular mechanisms that regulate experience-dependent DVB remodeling and spicule protraction behavior using candidate approaches. Recent work by other labs generated gene expression profiles of neurons in both sexes, allowing identification of genes that are differentially expressed in male vs hermaphrodite DVB - which could be potential candidates to function in male-specific DVB remodeling. Interestingly, multiple neuropeptides (NP) were amongst the DEGs, leading us to wonder if NPs function in DVB neuronal remodeling. NPs are secretory molecules that modulate neuronal activity and influence behaviors such as locomotion, appetite/satiety, and sleep. We identified nine candidate NPs that are more highly expressed in the male DVB neuron. Using endogenous transcriptional reporters, we confirmed the differential expression of two NPs in DVB, *flp-28* and *nlp-73*, and found that *flp-28* expression increases in DVB from day 1 to day 3 in males. To assess the overall impact of NPs on DVB neuroplasticity, we examined mutants with disruptions in NP synthesis and secretion pathways. Deletion of *egl-21*, a gene encoding a conserved NP-processing enzyme, led to increased neurite branch length and junction number in day 3 adult males. Similarly, *unc-31* mutants, which are defective in NP secretion, displayed an increase in neurite junction number. Additionally, *unc-31* mutants have an increased time to spicule protraction on aldicarb. Together, these results suggest a potential functional role of NPs in DVB neuronal remodeling. Currently, we are working on pan-neuronal and DVB-specific expression of *egl-21* to determine if NPs function in DVB remodeling in a cell-autonomous manner. We are also characterizing the expression patterns and functional impacts of the other candidate DVB NPs. These findings provide strong evidence that NPs contribute to experience-dependent neuronal remodeling, offering insights into their broader roles in both healthy and diseased brains.

752C Investigating the role of SSRIs' as a potential therapeutic for neurodegenerative diseases

Olivia Reed, Daniel M Hong, Alexey V Revtovich, Natasha V Kirienko Rice University

Neurodegenerative diseases (NDDs) are prevalent in the U.S., with chronic neurodegenerative conditions predicted to double in the next two decades. Many of these diseases share similar characteristics, including aggregate related pathologies and mitochondrial dysfunction. Proper mitochondrial recycling, via mitophagy, is crucial for maintaining mitochondrial homeostasis and overall neuronal health. Initiation of mitophagy requires the stabilization of PINK1 on the outer mitochondrial membrane. Previously, the Kirienko Lab screened 45,000 compounds for PINK1 stabilizers in *C. elegans* and identified sertraline as a promising candidate. Sertraline is an FDA-approved selective serotonin reuptake inhibitor (SSRI) commonly prescribed to treat depression, however it has also been shown to increase survival and neurogenesis in mammalian Huntington's and Parkinson's disease models. In our study, we investigated whether other FDA-approved SSRIs could alleviate neurodegenerative symptoms.

Our follow-up experiments showed that sertraline increased motility, average distance traveled, and rotation index in *C. elegans* expressing β-amyloid in body wall muscle cells. Moreover, it decreased protein aggregation in Alzheimer's, Huntington's, and Parkinson's disease models, as well as age-dependent aggregation of non-pathogenic LBP-2 protein. Testing additional SSRIs showed that some, but not all, had these neuroprotective effectives. Supplementation with serotonin did not affect phenotypes, but inhibition of mitophagy abolished the rescue, suggesting these molecules are moonlighting, with some function in mitochondrial metabolism. Treatment with cobalamin (vitamin B12), a critical cofactor in mitochondrial branched-chain fatty acid metabolism, or metformin, a compound known to improve mitochondrial function, alleviated paralysis in the Alzheimer's disease model, corroborating the relationship between improved mitochondrial health and alleviation of NDD symptoms. Our results reveal the potential of repurposing SSRIs as a therapeutic for NDDs.

753C The Loss of Mojo Project: Leveraging the Variable Decay of Mating Prowess in *C. elegans* for Behavioral Genetics

Gregory Reilly, Eric Haag University of Maryland

In a selfing hermaphroditic nematodes such as *C. elegans*, maintaining an ideal ratio of males in a population presents a key evolutionary issue. Too many males reduce population growth [1] while too few males prevent outcrossing, a vital tool for adapting to environmental changes [2]. Therefore, a balance must be struck in this ratio; this balance may be reflected in the mating behavior variation of *C. elegans*. The common lab strain of worms, N2, has males that are relatively poor maters, while a Hawaiian strain, CB4856, has substantially better male maters [3]. However, mating behavior has only been rigorously examined within the N2 strain. Furthermore, the role hermaphrodites have in the variation of mating success remains inadequately understood. We hypothesize that while selection on mating behavior has been relaxed across the species, the resulting degradation is variable across global diversity. We further hypothesize that factors underlying this variation may be mappable with modern methods for phenotyping and genotyping. This approach could complement forward genetics screens as a way to discover new factors affecting behavior and the neural systems underlying it. We began by examining the persistence of males in the diverse set of strains from CaeNDR. This simple assay integrates both pre-copulatory and post-copulatory aspects of mating. We found that our strain set fell into three groups: high male persistence (good maters), middling male persistence (ok maters), and little to no male persistence (poor maters). To further examine variation in mating behavior, we developed a male mating efficiency assay as well as a hermaphrodite mating efficiency assay to quantify changes in mating behavior. This work seeks to further elucidate the relationship between genetics and behavior in ecological variation.

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754C The role of gap junction channel INX-19 in nervous system function across the lifespan

Molly Reynolds[1], HaoSheng Sun[21]Cell, Developmental Integrative Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

Electrical synapses, formed by gap junction channels composed of connexins/innexins, are dynamically regulated throughout development and play critical roles in nervous system function across the lifespan. Despite the identification of neuronal gap junction defects in neurological disease, the mechanisms regulating gap junctions and their roles across development and diseases remain largely unknown. Here, we investigate the neuronal-specific gap junction protein INX-19 (*innexin-19)* in *Caenorhabditis elegans* to elucidate the regulation and functional roles of dynamic neuronal gap junction expression throughout life. First, we characterized *inx-19* expression pattern at single-neuron resolution from hatch to aged adults, observing a significant decrease across development, particularly between late larval stages and adulthood. Concurrently, we are identifying genetic regulators that control the temporal expression of *inx-19* across the lifespan. Next, to examine the functional roles of *inx-19*, we analyzed genetic null mutants and tracked locomotion across the lifespan. Surprisingly, despite the highest *inx-19* expression during embryonic and early larval stages, *inx-19* mutants showed the most dramatic locomotion defects in middle-aged (Day 5) adults, suggesting premature nervous system aging. We further investigated the critical periods for this early aging phenotype by spatiotemporally depleting *inx-19* during embryonic, larval, or early adult stages using the auxin-inducible degron (AID) system. Lastly, to further support our hypothesis that *inx-19* mutation contributes to premature aging and neurodegeneration, we depleted *inx-19* in tauopathy neurodegenerative mutants and found exacerbated locomotion deficits in aged adults. Altogether, our findings reveal a novel role for *inx-19* in neuronal aging and neurodegeneration, providing a platform to further dissect underlying mechanisms. This work enhances our understanding of how dysregulated dynamic electrical synaptic expression across the lifespan contributes to age-related neural decline and may inform novel therapeutic strategies.

755C Neurotoxic Impact of TMEM106B C-Terminal Co-Aggregation in *Caenorhabditis elegans*

Ruben Riordan[1], Aleen Saxton[2], Kailee Parrott[2], Rebecca Kow[2], Nicole Liachko[1], Brian Kraemer[11]GRECC, University of Washington / VAPSHCS, [2]University of Washington / VAPSHCS

Diseases such as Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Lewy Body Dementia (LBD) are complex neurodegenerative conditions caused by aggregation and dysfunction of pathogenic proteins like tau and α-synuclein. While these diseases are often studied under the context of a single aggregating protein, more often than not, disease involves co-aggregation of multiple pathogenic species. Thus far, the field has produced no effective disease modifying therapeutics for neurodegenerative disease, in part due to our lack of understanding how co-aggregating factors work together to drive neurotoxicity.

In early 2022, previously unidentified protein aggregates in the brains of frontotemporal lobar degeneration (FTLD) cases were determined to be made up of the C-terminal fragment of Transmembrane Protein106B (TMEM106B). Since then, aggregation of the C-terminal fragment of TMEM106B (TMEM-CT) has been observed in the brain in nearly every neurodegenerative disease as well as with aging in general. TMEM106B is a lysosomal transmembrane protein that has been associated with risk for most neurodegenerative diseases, though the mechanism had remained elusive. Under normal conditions, the TMEM-CT is believed to guide the protein to the lysosomal membrane before undergoing cleavage and lysosomal degradation. The discovery that the TMEM-CT aggregates in disease rather than undergoing lysosomal degradation represents a novel mechanism for TMEM106B neurotoxicity. However, the neurodegenerative potential of TMEM-CT aggregation as well as the impact of TMEM-CT co-aggregation with hallmark pathogenic proteins remained unknown.

Previously, we developed and characterized the first live organism model of TMEM-CT aggregation in Caenorhabditis *elegans* (*C. elegans*). We showed that all observable TMEM-CT was densely aggregated, and this aggregation resulted in severe neuronal dysfunction and significant neuronal loss, confirming the expectation that TMEM-CT aggregation can be neurotoxic. Now, to explore the effect of TMEM-CT co-aggregation with other pathologic neurodegenerative proteins, we co-expressed TMEM-CT with the hallmark pathogenic species of neurodegenerative disease: tau, α-synuclein, amyloid β 1-42 (Aβ 1-42), TAR DNA Binding Protein 43 (TDP-43), and poly-glutamine (poly-Q) in our *C. elegans* model. With these bigenic worms, we assessed synergistic effects of two aggregating proteins on neuronal function, neuron loss, and pathogenic protein accumulation.

756C Differential regulation and functional analysis of Major Sperm Proteins in ADL neurons

Isabel L Ross[1], David Greenstein[2], Sarah E Hall[11]Biology, Syracuse University, [2]Genetics, Cell Biology, and Development, University of Minnesota

C. elegans nematodes integrate a range of signals related to the quality of the environment primarily through amphid sensory neurons and use this information to decide whether to initiate continuous development or enter the developmentally arrested, stress resistant dauer diapause stage. Our lab has demonstrated that the ADL neurons of animals that pass through dauer due to crowding (postdauers) have distinct gene expression profiles from animals that develop continuously (controls). RNA-seq of isolated ADL neurons led to the identification of 116 and 595 genes that were up- or down-regulated, respectively, in wildtype postdauer animals compared to controls. Interestingly, among the group of downregulated genes were 145 genes that were characterized as spermatogenic, including 21 members of the *major sperm protein* (*msp*) family. In sperm, MSPs have dual roles as cytoskeletal proteins and secreted signaling molecules, but their functional significance in neurons is unknown. While MSPs themselves are unique to nematodes, they are derived from an ancestral protein family called VAP (VAMP Associated Protein) proteins that are conserved across metazoans. VAP proteins are type II ER membrane proteins with a conserved N-terminal MSP domain (MSPd), which interacts with the mitochondrial membrane-anchored protein PTPIP51 to promote calcium exchange and lipid transfer. In addition, the MSPd is cleaved from the other domains and secreted from the cell to signal to other tissues. Although MSPd cell non-autonomous functions are not well-studied, they are implicated in neurodegenerative diseases such as ALS and Alzheimer's Disease. To investigate the function of MSPs in the nervous system, we are investigating the function of *C. elegans* VAPB homolog, VPR-1, in sensory neurons. We have found that loss of the *vpr-1* MSPd results in the failure of animals to respond to the odorants diacetyl and benzaldehyde. Although *vpr-1* mutants were previously characterized as *maternal effect sterile*, we have shown that homozygous *vpr-1* mutants (M+/Z-) have a diet-dependent sterility: *vpr-1* animals are 99% sterile on *E. coli* OP50, and 28% sterile on NA22. These results suggest that the mutant phenotype of *vpr-1* may be modulated by diet. Given the known importance of the MSPd in neurons, and the unexpected finding that MSPs are expressed in ADL in *C. elegans*, we will develop this system as a model to investigate the functional significance of MSPs and MSPd-containing proteins in neurons.

757C Regulatory Roles of NID-1/Nidogen in Pioneer Axon Navigation in the Ventral Nerve Cord

Debapriya Roy, Tianna Martin, Jae Riley, Harald Hutter Biological Sciences, Simon Fraser University

Pioneer axons establish the initial axon tracts in the developing nervous system, providing a scaffold for later follower axons to reach their targets. The basement membrane (BM) protein NID-1/Nidogen is crucial for proper axon navigation in the ventral nerve cord (VNC) [Kim & Wadsworth et al., 2000]. We found that mutations in several genes exacerbate AVG pioneer axon defects in a *nid-1* mutant background, suggesting that NID-1 interacts with multiple axon guidance pathways [Feresten AH et al., 2023]. Additionally, *nid-1* mutants exhibit highly penetrant defects in PVPR pioneer axon navigation, including the failure of PVPR to pioneer the left VNC and ventral midline crossing of PVPR axons. These findings indicate that NID-1 plays a key role in establishing pioneer axon trajectories. NID-1 is a multimodular protein composed of three globular domains: G1/NIDO, G2, and G3 linked by rod-like domains containing epidermal growth factor (EGF)-like repeats. It interacts with key BM components: laminin binds to G3, collagen IV associates with both G2 and G3, and perlecan binds to G2. Imaging of *nid-1* strain tagged with mNeonGreen [Keeley et al., 2020] reveals its presence in the BM surrounding the VNC, with enrichment near the right and left axon tracts. To dissect the role of individual NID-1 domains in VNC axon navigation, we used CRISPR/Cas9 to generate two deletion alleles: *nid-1(hd189),* which lacks the G2, EGF, and G3 domains, and *nid-1(hd188),* which lacks only the G3 domain. Phenotypic analysis showed that *nid-1(hd189)* resulted in severe PVPR and PVQL axon navigation defects, with PVPR pioneering defects (80%) comparable to those of the null allele (83%) but more penetrant than *nid-1(cg118)* (58%), which lacks the G1 and G2 domains. In contrast, deleting only the G3 domain *nid-1(hd188)* led to reduced penetrance of the overall defect (64%) compared to the null allele (95%) and a lower penetrance of PVPR pioneering defects (19%). These findings suggest that the G2 and EGF domains are most important for NID-1's role in PVPR axon navigation. We are currently examining whether the AVG pioneer axon is equally dependent on particular domains. We hypothesize that NID-1 influences axon guidance either directly or indirectly through interactions with other proteins. To further elucidate its function, we will investigate known NID-1 interaction partners for a role in pioneer axon navigation.

758C Inheritance of probiotic-mediated neuroprotection in a model of Amyotrophic Lateral Sclerosis

Rocio R Rubiano, Jie Ren, Yu Sang, Alejandro Aballay Genetics, The University of Texas MD Anderson Cancer Center

Amyotrophic Lateral Sclerosis (ALS) is a severe, adult-onset neurodegenerative disorder with a life expectancy of 3-5 years upon diagnosis. While most ALS cases are believed to be sporadic, the rise in genetic testing in the United States has led to an increase in the number of familial ALS cases with a known genetic driver mutation. In the US, the most prominent mutation seen in the ALS patient population is an alanine to valine substitution in the copper-zinc superoxide dismutase protein 1 (SOD1 A4V). The SOD-1 A4V mutation disrupts reactive oxygen species handling and leads to proteinaceous accumulations that ultimately result in oxidative stress-induced loss of cholinergic motor neurons. Among the many health-promoting activities of the bacteria leveraged in probiotics, Gram-positive bacteria exhibit antioxidant properties. *E. faecium* in particular has been reported to support the immune system, reduce signs of oxidation in the brain, and increase antioxidant enzyme activity. Utilizing a *C. elegans* single copy knock-in model of the SOD-1 A4V mutation that highly recapitulates the human disease phenotype, we found that probiotic exposure prior to oxidative stress has a significant neuroprotective effect. Young adult SOD-1 A4V animals were allowed to feed on either an *E. faecium* lawn or *E. coli* for 24 hours. After probiotic exposure, a synchronized population of progeny was obtained and allowed to grow up to young adults on *E. coli*. The animals were exposed to paraquat for 24 hours to induce oxidative stress, at which point they were imaged and scored for neurodegeneration. Neurodegeneration was considered loss of two or more cholinergic motor neurons in the posterior ventral nerve cord. The progeny of probiotic-exposed animals exhibited retention of the posterior cholinergic motor neurons upon oxidative stress, indicating a neuroprotective effect when compared to control animals. This suggests that the neuroprotective effect of probiotic treatment can be maintained through inheritance without need for repeated, prolonged exposure.

759C Diet, Chemotherapy, and Neuropathy: Investigating Shared Molecular Pathways in a *C. elegans* Model

Manuel A Ruiz[1], Hongfei Ji[2], Chandni Jaisinghani[2], Caroline Arellano-Garcia[2], Miriam B Goodman[31]Molecular and Cellular Physiology, Stanford School of Medicine, [2]Stanford School of Medicine, [3]Moelcular and Cellular Physiology, Stanford School of Medicine

Peripheral neuropathy (PN) affects approximately 13.5% of adults aged 40 or older in the U.S., with diabetes being a leading cause. Chemotherapy agents such as vincristine also increase the risk of PN, leading to chemotherapy-induced peripheral neuropathy (CIPN). When diabetes and vincristine exposure are combined, the risk of PN is further heightened. Despite extensive research, we do not know whether CIPN and diabetic peripheral neuropathy (DPN) share common molecular pathways or if there is any crosstalk between these conditions. Understanding these pathways and their relationship to one another is critical to developing targeted interventions.

C. elegans is an excellent model system for studying CIPN and DPN due to advanced genetics, conserved neurotransmitter and neuromodulator pathways, ease of quantitative behavioral studies and the integration of these features with RNA sequencing. Previous studies demonstrate that treating worms with a high-glucose diet to model DPN produces defects in motor and sensory-associated behaviors including touch response (Ruiz et al., unpublished). Additionally, it is known that high-glucose diet modifies the expression of genes required for neurotransmitter release and function (Garcia et al, 2015, Genetics 1:167-84). To learn more about the interaction of DPN and CIPN-like treatments, we will determine how a high-glucose diet alone compares to treatment with vincristine alone and to combination treatment. We will present data showing that these treatments seem to affect sensory behaviors independently of one another. It remains unclear, however, if the effects of high-glucose diets and vincristine are additive, which we will test using classical touch assays. In parallel, we will conduct RNA sequencing on glucose-fed animals, vincristine-treated animals, and glucose-fed animals treated with vincristine. This dataset has the potential to identify molecular pathways underlying these neuropathies that are shared and those that are distinct. Once candidate genes are identified, we will test mutants carrying defects in candidate genes for their effects on PN. Finally, we will seek to identify strategies that might rescue glucose-induced and vincristine-induced touch impairment. Our goal is to uncover shared or distinct genes driving CIPN and DPN, providing insight into their pathogenesis and potential therapeutic targets.

760C *srrt-1* loss of function rescues proteinopathy in *C. elegans* models of neurodegenerative disease

Aleen Saxton1, Randall Eck1,2, Asia Beale1, Rebecca Kow1,3, Brian Kraemer1,3,4,51Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA., 2Neuroscience, Graduate Program in Neuroscience, University of Washington, Seattle, Washington 98195, 3Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA 98104, USA., 4Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98195, 5Department of Laboratory Medicine & Pathology, University of Washington, Seattle, Washington 98195

Neurodegenerative diseases affect approximately 15% of the world population, causing a variety of cognitive and physical disabilities. Among these neurodegenerative diseases are amyotrophic lateral sclerosis (ALS), a debilitating, fatal disease that causes rapid muscle wasting, and Alzheimer's disease (AD), characterized by cognitive decline and memory loss associated with accumulation of tau tangles and beta-amyloid plaques. Mutations in the human gene encoding TDP-43 cause ALS in a subset of cases and wild-type TDP-43 behaves aberrantly in many neurodegenerative diseases, including correlating to more severe cases of AD.

To understand the molecular drivers of neurodegeneration, we have modelled several human disease pathologies in *Caenorhabditis elegans*, a simple animal model advantageous for its short lifespan, ease of cultivation, thoroughly characterized nervous system, and well-established behavioral assays. We have previously generated and published a variety of transgenic *C. elegans* models of ALS- and AD-related proteinopathies by overexpressing human TDP-43 or human tau with or without disease causing mutations.

Via a forward genetic screen utilizing EMS mutagenesis, we identified a genetic suppressor of TDP-43 proteinopathy that improves motor function, decreases accumulation of toxic aggregating proteins, and rescues neurodegeneration. The gene of interest, *srrt-1*, codes for a highly conserved protein originally identified for its arsenite resistance conference in plants and established as arsenite-resistance protein 2 (ARS2) or serrate RNA effector molecule homolog (SRRT) in humans. SRRT-1 in *C. elegans* is a nuclear protein involved in RNA quality control and transcript maturation through its interactions with the cap-binding complex (CBC).

Loss of SRRT-1 leads to a reduction in disease relevant phenotypes in both tau and TDP-43 proteinopathy models of neurodegeneration. Here, we will present the ongoing characterization of the complex interaction between human TDP-43, human tau, and *C. elegans* SRRT-1 with discussion of the molecular mechanisms contributing to neurodegeneration.

761C Optogenetic inhibition of glutamatergic transmission by clustering of synaptic vesicles

Noah Schuh, Dennis Rentsch, Alexander Gottschalk Cellular and Molecular Neurobiology, Johann Wolfgang Goethe-University Frankfurt

Understanding the function and connectivity of neurons, as well as the molecular mechanisms of synaptic transmission and its modulation, is crucial for advancing neuroscience. Optogenetic tools provide precise, dynamic control over neuronal activity, enabling investigation of specific neuronal roles and synaptic processes. The optoSynC (optogenetic synaptic vesicle clustering) tool (PMID: 36535932), silences synaptic transmission by inducing light-evoked clustering of synaptic vesicles (SVs) via the homo-oligomerization of the *Arabidopsis thaliana* cryptochrome protein CRY2. This fast, reversible, and non-disruptive approach prevents SV availability for neurotransmitter release without altering ionic currents, cellular biochemistry, or synaptic protein composition. OptoSynC has been validated across multiple model systems, demonstrating its versatility.

To further explore and enhance the applications of the optoSynC platform, we developed “glutamate exclusive (GluE)-optoSynC”, a modified version tailored to selectively silence glutamatergic transmission in *C. elegans*. In this version, the general synaptic vesicle protein synaptogyrin (SNG-1) was replaced by the vesicular glutamate transporter EAT-4 as the optoSynC SV-anchor. This modification enables GluE-optoSynC to specifically cluster glutamatergic SVs and achieve light-induced, neurotransmitter-specific synaptic silencing. Additional GluE-optoSynC variants combined CRY2 and the N-terminal portion of the CRY2-interacting protein CIB1 (CIBN) with EAT-4, providing different vesicle clustering configurations.

We assessed GluE-optoSynC performance by assaying behaviors depending on glutamatergic transmission, including food-leaving, hyper-foraging, pharyngeal pumping, and anterior touch response — behaviors defective in *eat-4(ky5)* mutants with impaired vesicular glutamate loading and release. In wild-type animals, light-induced GluE-optoSynC activation led to behaviors resembling those of *eat-4(ky5)* mutants, and in mutants expressing GluE-optoSynC, partial behavioral restoration occurred due to functional EAT-4 incorporation. Blue light activation suppressed this restored behavior, demonstrating reversible modulation of glutamatergic signaling. We are currently testing GluE-optoSynC in neurons mediating behaviors depending on glutamate and a second transmitter, to assess specific suppression of just the glutamate-dependent output.

Our findings establish GluE-optoSynC as a promising tool enabling neurotransmitter-specific targeting. Despite performance variability across assays, GluE-optoSynC provides an effective method for acute, reversible silencing of glutamatergic transmission and opens possibilities for silencing other neurotransmitter-specific transmissions.

762C Phosphatidylinositol (4,5)-bisphosphate Impacts Ectosome Shedding from *C. elegans* Ciliated Sensory Neurons

Alexis Semmel, Malek Elsayyid, Nahin Siara Prova, Krisha D Parekh University of Delaware

Small secreted extracellular vesicles (EVs) mediate the intercellular transport of bioactive macromolecules during physiological processes and propagation of pathological conditions. The primary cilium, a sensory organelle protruding from most non-dividing cells, transmits signals by shedding EVs called ectosomes. The periciliary membrane compartment (PCMC) at the cilia base is separated from the cilium proper by the transition zone (TZ), which acts as a gate to confine proteins and lipids into the different compartments. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) is concentrated in the plasma membrane and PCMC, while phosphatidylinositol 4-phosphate PI(4)P is found at high levels in the cilium proper. Given the known impacts of PI(4,5)P2 on protein localization, actin dynamics, endocytosis, exocytosis, and membrane deformation, we reasoned that the ciliary compartmentalization of PI(4,5)P2 could have functional relevance for ectosome biogenesis. We found that the C. elegans type I phosphatidylinositol 4-phosphate 5-kinase PPK-1 localizes to and regulates PI(4,5)P2 abundance in the ciliary base, while the C. elegans phosphoinositide 5-phosphatase INPP-1 localizes to and impacts PI(4,5)P2 in the cilium proper of male-specific ray type B (RnB) EV-releasing neurons. Through overexpression of PPK-1 and deletion of INPP-1, we manipulated PI(4,5)P2 levels in the RnB neurons, then determined the impact on shedding of ectosomes carrying cargos tagged with fluorescent proteins. We discovered that high PI(4,5)P2 differentially impacted shedding of two distinct EV subpopulations, decreasing biogenesis of ectosomes derived from the PCMC, but increasing budding from the cilium distal tip. Reduced EV shedding from the PCMC was correlated with lower abundance of the EV cargo in the ciliary base, suggesting that endocytosis and ectosome budding from the PCMC are coupled to regulate protein content. In contrast, an increase in ciliary PIe(4,5)P2 did not cause an overall change in cargo abundance within the cilium proper or the length of the cilium. This suggests that increasing PI(4,5)P2 in the cilium proper can serve as a mechanism to enhance EV ectocytosis from the distal tip without altering cilium morphology.

763C The SCRM-1 Phospholipid Scramblase Regulates Shedding of Extracellular Vesicles

Alexis Semmel University of Delaware

Small secreted extracellular vesicles (EVs) that contain bioactive molecules are shed by most, if not all, cell types. It is important to identify mechanisms of EV biogenesis in order to understand how this type of intercellular communication is regulated to impact physiological and pathological processes. EVs can be released from primary cilia, microtubule-based organelles that protrude from the cell surface. In C. elegans, EVs bud from the non-motile cilia of sensory neurons and are released into the environment through pores in the cuticle. We previously discovered that the CLHM-1 ion channel is a cargo in EVs released from the periciliary membrane compartment (PCMC) at the ciliary base, while the polycystin TRP channel PKD-2 is present in EVs shed from the cilium distal tip. Our goal is to determine how phosphatidylserine (PtdSer) exposure impacts the biogenesis of ciliary-derived EVs. Two proteins work to maintain or disrupt PtdSer asymmetry, the flippase TAT-1 and the scramblase SCRM-1, respectively. Flippases use ATP to actively "flip" PtdSer from the outer leaflet to the inner leaflet, while scramblases can flip phospholipids in both directions. Notably, activation of SCRM-1 has been shown to lead to externalization of PtdSer. We discovered that SCRM-1 localizes to the PCMC and transition zone in the RnB neurons. Using C. elegans that express CLHM-1 tagged with tdTomato and PKD-2 tagged with GFP at single copy we then sought to determine if loss of scrm-1 impacts EV biogenesis of the different EV subpopulations shed from the male tail RnB neurons. Deletion of scrm-1 caused a significant decrease in shedding of CLHM-1 EVs while the number of PKD-2 EVs was unaffected. Analysis of CLHM-1::tdT localization in the RnB neurons showed that the amount of CLHM-1 was reduced in the ciliary base. This suggests that regulation of PtdSer asymmetry in the PCMC can be used to specifically regulate cargo abundance and biogenesis of the EVs that are shed from this location. We are currently performing experiments using a tat-1 null mutant to further determine how PtdSer plays a role in EV biogenesis from the primary cilium.

764C Neuronal sexual dimorphism revealed by single-cell transcriptomics

Hagar Setty[1], Rizwanul Haque[2], Ramiro Lorenzo[3], Gil Stelzer[4], Yehuda Salzberg[1], Gal Goldman[1], Elad Schneidman[1], Patrick Laurent[3], Meital Oren-Suissa[11]Brain Sciences, Weizmann Institute of Science, [2]Division of Medical Research, SRM Institute of Science and Technology, [3]ULB Neuroscience Institute, [4]Life Sciences and Core facilities, Weizmann Institute of Science

The molecular mechanisms that underlie differences between the sexes in sex-shared connectivity, circuit function, and behavior remain largely unexplored. To what extent did the sex-shared nervous system diverge to utilize distinct genes and molecules between the two sexes?

To answer this question, our lab generated a single-cell RNA-sequencing dataset of the entire nervous systems of hermaphrodites and males. These transcriptomic profiles revealed extensive sexual dimorphism in gene expression of multiple neuronal gene families, with a prominence for neuropeptides encoding genes showing a male-biased expression. We discovered that most sex-shared neurons in our dataset share the same terminal selectors and neurotransmitter identity features, suggesting that dimorphism is encoded in other neuronal regulatory elements. However, we did find sexually dimorphic expression of some neurotransmitter-related genes, indicating differences in neurotransmitter release.

By ranking neurons based on the degree of molecular dimorphism, we identified sex-shared neurons previously unknown to be dimorphic, among them the posterior touch receptor neuron PLM. Importantly, we discovered that the response of males to posterior gentle touch is greater than that of hermaphrodites, and that PLM basal activity is higher in males, demonstrating that the dimorphic transcriptional profile of PLM translates into behavioral differences between the sexes. Silencing the hermaphrodite-enriched RNA-binding protein MEC-8 reduced posterior touch sensitivity in hermaphrodites only.

We further utilized our dataset of both sexes and the connectome of *C. elegans* to generate predictions on the identity of genes that regulate synaptic wiring. We calculated the correlation between the number of outgoing connections and the average gene expression values of all genes in our dataset. We discovered 54 genes with significant correlation in both sexes, including known neuronal genes as well as genes that haven't been previously associated with connectivity. We harnessed this list of genes and found *ttx-7,* which encodes a Myo-inositol monophosphatase (IMPase), and *zig-1* predicted to encode a cell adhesion molecule, regulate synapse localization in a cell- and sex-specific manners.

Overall, our work uncovers novel dimorphic molecular landscapes and functions for sex-shared neurons and provides an important framework to explore how genetic sex modulates gene expression to shape behavioral outcomes.

765C Glial regulation of sensory processing and sleep in *C. elegans*

Liza J Severs[1], Sean R Manning[1], Maria D Purice[2], Aakanksha Singhvi[11]Basic Sciences Division, Fred Hutchinson Cancer Center, [2]Biochemistry and Biophysics, Oregon State University

Neurons and glia are the two major brain cell types, where glia regulate neuronal properties and circuit functions, thereby impacting animal behavior. In *C. elegans*, glia reside in sense organs and regulate and respond to sensory stimuli across modalities. For example, AMsh glia in the amphid sense organ modulate the animal's ability to sense its environment and have been shown to impact temperature and odorant sensation through its partner sensory neurons. In the case of particular odorants, the AMsh autonomously responds to these cues. Preliminary findings indicate that calcium fluctuations occur in multiple glia in animals experiencing a temperature gradient. Global dampening of sensory responses is a hallmark of sleep across species. However, how glial sensory processing corresponds with sleep has not been systematically evaluated. My research aims to dissect the molecular pathways by which glia integrate sensory processing and sleep regulation, using *C. elegans* as a model.

C. elegans exhibit canonical sleep characteristics like in other organisms with reversible quiescent behavior, an increased arousal threshold for sensory stimuli, characteristic changes in posture, and a homeostatic response to sleep deprivation. The circadian clock gene *lin-42*, a homolog of mammalian PERIOD, regulates the animal's sleep state. Previous work has shown that LIN-42 regulates animal molt cycles. From our group's recently published glia molecular profiling atlas (wormglia.org), I observed that LIN-42 is expressed in multiple glial subtypes. I also found that age-matched *lin-42* mutant animals have severely impaired thermotaxis behavior. Thus, LIN-42 is expressed in glia, and regulates both animal sleep and sensory responses. Using this as a molecular tool for mechanistic insight, my ongoing experiments are investigating the role of glial LIN-42 in thermosensory responses and sleep.

Independently, we leveraged insights from our glia atlas to develop GliaPAL, a transgenic animal with all glia reproducibly labeled. Thus, in a corollary approach, my aim is to couple GliaPAL with pan-glial calcium imaging to determine how activity of glia across the nervous system tracks animal sleep states.

766C EZ-FRCNN: A fast, accessible, and robust deep learning package for tracking *C. elegans*

Erin Shappell[1], Jacob Wheelock[1], Guillaume Aubry[2], Hang Lu[21]Bioengineering, Georgia Institute of Technology, [2]Chemical and Biomolecular Engineering, Georgia Institute of Technology

Advances in automation and throughput of neurobiological and behavioral studies in *C. elegans* have consequently enabled the collection of large-scale datasets. Due to the increased size and complexity of these datasets, the demand for dataset labeling has increased accordingly. While expert-driven manual labeling is a standard in *C. elegans* research, this growing demand for labeled data has made manual labeling impractical due to the infeasible time requirements. Furthermore, using machine learning methods for automated labeling often requires a programming background. To widen the accessibility of reliable automated labeling to biologists with a limited programming background, we introduce EZ-FRCNN: a locally hosted application designed to provide researchers with Faster R-CNN (Region-based Convolutional Neural Network) tracking via a user-friendly interface that requires no programming experience. This all-in-one package allows users to annotate, train, validate, and apply Faster R-CNN models to label any dataset, even under challenging imaging conditions. We demonstrate the robustness and flexibility of EZ-FRCNN via applications to tracking the grinder in freely moving worms and tracking freely swimming worms in droplets. These applications highlight EZ-FRCNN's ability to enhance biological research by providing scientists with an accessible and reliable implementation of a widely used machine learning tool that may be tuned for virtually any tracking project. This package will be made freely available via GitHub with instructions on how to install and run the program.

767C A heteromeric (LGC-47/ACC-1) inhibitory acetylcholine receptor gates context-dependent mechanosensory processing in *C. elegans*

Sandeep Kumar[1], Anuj Sharma[2], Andrew Leifer[31]Princeton Neuroscience Institute, Princeton University, [2]Department of Physics, Princeton University, [3]Department of Physics & Princeton Neuroscience Institute, Princeton University

An animal's current behavior influences its response to sensory stimuli, but the molecular and circuit-level mechanisms of this context-dependent decision-making are not well understood. Previously we discovered that *Caenorhabditis elegans* is less likely to respond to mechanosensory stimuli by reversing if the stimulus was received while the animal was in the middle of a turn (Liu *et al.* 2018; Liu *et al.* 2022). We found that inhibitory feedback from turning associated neurons is required to gate the reversal response (Kumar *et al.*2023). However, until recently, it has remained unknown precisely where in the circuit gating occurs, and which specific neurons and receptors receive the inhibitory feedback to perform the gating. Here we use genetic manipulations, single-cell rescue experiments and our high-throughput closed-loop optogenetic perturbation-during-behavior instrument (Liu *et al.* 2022) to reveal the specific neuron and receptor responsible for receiving inhibition and altering sensorimotor processing. Our measurements show that a heteromeric inhibitory acetylcholine-gated chloride channel comprising LGC-47 and ACC-1 expressed in neuron type RIM disrupts mechanosensory evoked reversals during turns, presumably in response to inhibitory signals from turning-associated neuron SAA (Kumar *et al.* 2024).

Our proposed circuit spans from motor to sensory and back. Specifically, inhibitory feedback from motor-related turning neurons is combined with downstream mechanosensory signals at a single interneuron pair by a single ion channel to gate the animal's motor response to stimuli. This convergence at a single receptor on a single neuron pair is striking and may reflect the unique constraints imposed by the worm's small nervous system of only 302 neurons. Even so, the broad approach of combining motor feedback with sensory signals to modulate a sensorimotor response is, in principle, accessible to many organisms and may therefore represent a general feature of context-dependent decision-making.

768C Distributed sensory circuit of pheromone avoidance is regulated by neuropeptides and neurotransmitters

Shruti Shastry Biology & Biotechnology, Worcester Polytechnic Institute

Sexually dimorphic behaviors in *Caenorhabditis elegans* are influenced by various molecular mechanisms, including the regulation of neuropeptides, which are short chains of amino acids that modulate neuronal activity and behavior. Our lab has been investigating the role of neuropeptides in mediating sex-specific responses to the pheromone ascaroside #8 (ascr#8). We found that males exhibit a strong attraction to ascr#8, while hermaphrodites demonstrate an avoidance response. These contrasting behaviors are regulated by distinct neuropeptide genes. Specifically, the neuropeptide gene *flp-3* was identified as a key modulator of male-specific attraction to ascr#8, indicating that its expression or activity is critical for driving this sexually dimorphic behavior. Conversely, the avoidance behavior observed in hermaphrodites is mediated by the neuropeptide genes *nlp-8* and *nlp-3*. The involvement of these genes suggests that hermaphrodites utilize specific neuropeptide signaling pathways to process and respond to ascr#8 as a repellent cue. Additionally, we identified a role for serotonin signaling in regulating the avoidance behavior. The enzyme tryptophan hydroxylase (*tph-1*), essential for serotonin biosynthesis, was found to influence the avoidance response, implying that serotonin serves as a modulatory signal in this behavioral pathway. Overall, our findings highlight the complex interplay between neuropeptide signaling and neurotransmitter pathways in governing sexually dimorphic behaviors in *C. elegans*, offering insights into the molecular basis of sex-specific responses to environmental cues.

769C Probiotic-Mediated neuroprotection via the gut-brain axis: Amelioration of neurodegenerative pathology in *C. elegans* models

YongWoon Shin[1], Jiaoyang Li[1], ShinSik Choi[21]Food and Nutrition, Myongji University, Republic of Korea, [2]Food and Nutrition, Bio-Pharmaceutical Sciences, Myongji University, Republic of Korea

Neurodegenerative disorders present significant healthcare challenges due to their complex pathophysiology and progressive neuronal degeneration. Recent advances in gut-brain axis research have revealed promising opportunities for microbiome-targeted interventions. Our research explores the neuroprotective potential of probiotics in both Parkinson's disease (PD) and Alzheimer's disease (AD) models. In our initial study targeting Parkinson's disease, we systematically screened probiotics from fermented foods for their ability to produce L-DOPA from L-tyrosine. We identified three effective strains that significantly restored dopamine-dependent behaviors in *Caenorhabditis elegans cat-2* mutants deficient in dopamine biosynthesis. These behaviors included basal slowing response, thrashing movements, and ethanol preference. Mechanistic investigations revealed that these probiotics upregulated the bas-1 gene encoding L-amino acid decarboxylase, effectively compensating for dopamine deficiency. Furthermore, they attenuated dopaminergic neuron degeneration and reduced pathological α-synuclein aggregation in the 6-OHDA neurotoxicity model. Building on these promising findings, we have expanded our research to investigate similar probiotic approaches for Alzheimer›s disease. Given that AD pathology involves abnormal amyloid-beta (Aβ) accumulation and that gut microbiota dysbiosis may facilitate Aβ entry into the brain through compromised barriers, we developed a *C. elegans* AD model expressing the human Aβ gene. This model enabled systematic screening of probiotic strains for their ability to ameliorate AD-related phenotypes. Preliminary observations indicated alterations in innate immunity pathways in our AD model, suggesting potential interplay between Aβ pathology and immune responses that may be modulated by probiotic interventions. Our ongoing research aims to elucidate strain-specific effects and underlying genetic mechanisms of neuroprotection. This work establishes a foundation for developing microbiome-based therapeutics for neurodegenerative disorders. By identifying specific probiotic strains with demonstrated efficacy in multiple disease models, our research may contribute to novel complementary strategies for managing these challenging neurological conditions.

770C Acidifying Lysosome pH by an Optogenetic Tool Improves Animal Fitness in a Neurodegenerative Disease Model

Shunpan Shu, Molly Hodul, Edwina Mambou, Aimee Kao Neurology, University of California, San Francisco

Aging is a major risk factor for neurodegenerative diseases. Despite emerging findings, there are still effective therapeutics for neurodegenerative diseases and incomplete understanding of its pathogenesis. Lysosomal pH has been shown to increase in *C. elegans* with age, altering its proteolytic efficiency and potentially contributing to neurodegenerative disease pathogenesis. We hypothesized that restoring lysosomal acidity could mitigate the accumulation of neurodegenerative proteins such as tau in neurons. To test this, we developed Acid-opHLy (Acidifying optogenetically pH of Lysosomes), an optogenetic tool in *C. elegans* that directly pumps protons into lysosomes while enabling real-time measurement and manipulation of lysosomal pH using ~540 nm light stimulation. A *C. elegans* model of tau overexpression exhibits developmental delays, reduced brood size, and impaired cellular homeostasis. Using a neuronal specific Acid-opHLy line, optogenetic acidification of neuronal lysosomes significantly increased the developmental rate from 30% to ~70% and brood size from ~10 to ~120. Together, our findings suggest that acidification of neuronal lysosomes improves its function and is protective against Tau overexpression. These findings highlight restoration of lysosomal aciditiy as a potential therapeutic strategy for neurodegenerative diseases.

771C ngn-1/Neurogenin functions in Insulin-dependent arrest

Alyssa C Sipman, Bruce Wightman Biology, Muhlenberg College

Insulin signaling promotes the progression of development. We have previously shown that the transcription factors *fax-1* and *unc-42* are potentiators of insulin signaling that promote progression to hatching and L1. Mutations in either transcription factor, in combination with a mutation in *daf-2* insulin receptor, result in a novel phenotype, called peri-hatching arrest, in which embryos arrest development in the egg or immediately after hatching in L1. *ngn-1* encodes the bHLH transcription factor neurogenin and is required for axon outgrowth, nerve ring formation, and neuronal specification. *ngn-1* is proposed to be upstream of *fax-1* and *unc-42*, as *ngn-1* mutants have decreased transcript levels as compared to the wild type. This relationship suggested that *ngn-1* mutations may also cause peri-hatching arrest. Indeed, *ngn-1; daf-2* double mutants appear to exhibit peri-hatching arrest at the same frequency as *fax-1; daf-2* double mutants, suggesting that the insulin potentiation function depends on *ngn-1*. An osmotic stress pathway, which depends upon the *ssu-1* cytosolic sulfatase, opposes the insulin signaling pathway. Mutations in *ssu-1* suppress the *fax-1; daf-2* peri-hatching arrest, and do the same for *ngn-1; daf-2* double mutants. These results expand the pathway for neuronal inputs into insulin signaling and regulation of developmental arrest and progression in the late embryo. By elucidating the role of *ngn-1* in insulin signaling and osmotic stress, this work will contribute to an understanding of how neuroendocrine pathways regulate developmental progression and may have implications for human neurological and developmental disorders.

772C Investigating the roles of *zoo-1/TJP* in synapse patterning in *C. elegans*

Andrew Snow, Sydney Ko, Olivia McCurry, Kota Mizumoto The University of British Columbia

Proper nervous system function depends on precise regulation of synapse number, arrangement, and target recognition. While many extrinsic cues that spatially control synapse formation have been elucidated, the intracellular effectors of these pathways remain poorly understood. Zonula occludens (ZO) proteins are cytoplasmic scaffolding proteins encoded by the *TJP* genes that link transmembrane proteins to cytoplasmic proteins and the actin cytoskeleton at intercellular junctions. Recent studies in zebrafish showed that ZO proteins promote the formation of electrical synapses by localizing Connexins. However, the role of ZO proteins in chemical synapse formation is not understood. The cholinergic DA9 motorneuron of *C. elegans* forms approximately 20 *en passant* synapses onto the posterior dorsal body wall muscles within a small subaxonal region. We and others have shown that the position of DA9 synapses is regulated by a prosynaptogenic cue, Neurexin, and the antisynaptogenic cues Plexin, Frizzled, and Netrin. In the loss of function mutant of *zoo-1*, the sole ortholog of TJPs in *C. elegans*, we observed a loss of synapses in the posterior synaptic domain and ectopic synapse formation in the anterior asynaptic domain, resulting in an anterior shift of the DA9 presynaptic domain. Interestingly, the ectopic synapse formation in the anterior asynaptic domain of *plx-1/Plexin* mutants, which is more severe than in *zoo-1* mutants, was partially suppressed by loss of *zoo-1*, suggesting that *zoo-1* may function downstream of *plx-1*. We previously found that PLX-1 negatively regulates RAP-2 activity to restrict synapse formation. As ZOO-1 and GTP-RAP-2 are both enriched in the presynaptic domain, we are investigating whether *zoo-1* may function with RapGEFs to regulate synapse formation. Intriguingly, we found that mutants of *srgp-1/srGAP* recapitulate the *zoo-1* phenotype, suggesting they may function together to regulate synapse formation. As SRGAP2 functions with SYNGAP1 and CTNND2 to regulate synapse density and maturation in mammals, we are testing the genetic relationship of *zoo-1* with *srgp-1*, *gap-2/SYNGAP1*, and *jac-1/CTNND2.* Currently, we are testing whether transgenic expression of ZOO-1 cDNA is sufficient to achieve a gain-of-function condition and to determine the tissues in which *zoo-1* functions. Together, our data show a novel role for *zoo-1* in mediating multiple signaling pathways to specify precise synapse patterning.

773C Functional analysis of APL-1/amyloid precursor protein (APP) signaling in the nervous system

Andrew Snow, Olivia McCurry, Kota Mizumoto The University of British Columbia

Amyloid Precursor Protein (APP) has been the subject of extensive research as the “Alzheimer’s Disease Gene”, yet its normal biological role remains poorly understood. APP intracellular domain (AICD), a γ-secretase cleavage product of APP, translocates into the nucleus to control gene expression, analogous to the mechanism of Notch signaling. However, the activity of APP signaling and its function in the nervous system are unclear. We investigate the role of APP signaling in the nervous system using *Caenorhabditis elegans,* which has a sole APP ortholog, APL-1. While null mutants of *apl-1* are lethal, mutants lacking the transmembrane and intracellular domains are viable (PMID: 17267616). Consistent with the previous work, we found APL-1 mutants lacking the transmembrane domain and AICD, or just the AICD, are hypersensitive to the acetylcholinesterase inhibitor aldicarb, suggesting a role for AICD in synapse function (PMID: 20862215; DOI: 10.1101/305284). We confirmed that transgenically expressed AICD::mStayGold can localize in the nuclei of neuronal cells. To visualize APL-1 signaling activity in vivo, we adapted a genetically encoded biosensor called SALSA developed for detecting Notch signaling (PMID: 35413239) and observed high APL-1 signaling activity in the nervous system. We found that the APL-1 signaling activity is reduced but not abolished in the *sel-12/Presenilin* mutant, suggesting there may be additional γ-secretases such as HOP-1 involved in the cleavage of APL-1. Currently, we are investigating the cell types in which APL-1 functions to control synapse function by conducting tissue-specific rescue experiments with APL-1 cDNA as well as conditional knockout experiments using a floxed *apl-1* allele we generated with CRISPR/Cas9 genome editing.

774C Exploring the metabolic basis of neurobehavioral defects associated with adenylosuccinate lyase deficiency

Sabrina Amita Sony, Wendy Hanna-Rose Biochemistry and Molecular Biology, The Pennsylvania State University

Adenylosuccinate lyase (ADSL) deficiency is a rare metabolic disorder characterized by muscle dysfunction and behavioral abnormalities, including autism-like symptoms. In *C. elegans, adsl-1* deficiency results in various phenotypes including impaired movement, sterility, altered learning, and aberrant regulation of head oscillations. ADSL enzyme deficiency leads to accumulation of its substrate SAICAR/r and a subsequent decrease in tyramine production, which causes the neurobehavioral phenotypes. To identify the mechanism by which SAICAR/r accumulation causes tyramine depletion, we first analyzed mRNA levels of *tdc-1,* which encodes the enzyme that produces tyramine from tyrosine, using qPCR. *tdc-1* mRNA levels are elevated in *adsl-1* mutant samples, suggesting that SAICAR/r does not result in transcriptional down regulation of *tdc-1*. We are currently examining TDC-1 enzyme activity. We are also exploring potential therapeutic interventions for ADSL deficiency. Perlara BioPharma, a company focused on repurposing approved drugs for rare diseases, conducted high-throughput screens and identified candidate drugs that rescued ADSL deficiency-related phenotypes in yeast. We are using *C. elegans* to validate efficacy and to deduce mechanism of action of these candidates. At least three candidates show ameliorating effects in the neurobehavioral assays: Disulfiram, 1-beta-D-Arabinofuranosyl Uracil (ARA-Uracil) and Fumarate. Future experiments will focus on determining the effects of the drugs on other phenotypes and employing LC-MS to analyze metabolite profiles in the presence of these compounds. With promising drug candidates in hand, we aim to build a molecular understanding of ADSL deficiency pathophysiology, ultimately paving the way for targeted therapeutic strategies.

775C Active forgetting is controlled via modulation of translational elongation

Attila Stetak University of Basel

Understanding neural circuits and molecular mechanisms underlying changes of synapse strength during learning and memory are the major challenges of neuroscience. However, not only learning and memory but also decay of memories (forgetting) is essential for proper functioning of the brain, and based on recent findings, forgetting similarly complex and regulated complex mechanism1-5 as learning and memory. Previously, we identified musashi (*msi-1*), an RNA-binding protein that actively regulates forgetting in *C. elegans*. *msi-1* regulates the Arp2/3 actin branching complex and acts downstream of GLR-1 AMPA-type glutamate receptor. The identification of a Musashi-dependent mechanism links translational repression to regulation of the structure of the actin cytoskeleton in neurons. To further investigate the molecular mechanism of MSI-1 in the regulation of translational control during forgetting we performed a yeast two-hybrid screen and identified among others EEF-2 as MSI-1 binding partner. We confirmed the initial finding with GST pull-down as well as with co-immunoprecipitation. In addition, we generated an integrated transgenic *C. elegans* line, expressing EEF-2 and MSI-1 proteins in AVA neuron, tagged with a tripartite split GFP system (EEF-2::spGFP10 and MSI-1::spGFP11) and we found that EEF-2 and MSI-1 forms a complex in AVA neuron that is localized in specific subcellular compartments likely representing synapses. EEF-2 activity is modulated by a unique mechanism involving EFK-1 a specific protein kinase. Phosphorylation of EEF-2 at specific threonine sites by the kinase inhibits its activity therefore based on our preliminary findings, deletion of *efk-1* could modulate memory in worms. To test this hypothesis, we analysed the memory phenotype of *efk-1(lf)* mutant and shown that removal of the EEF-2 kinase inhibited forgetting similar to deletion of *msi-1* but only during long-term memory. Using different inhibitors and activators of the EFK-1 pathway we also identified a molecular pathway that regulates forgetting. Altogether, our results demonstrate that MSI-1 likely regulates forgetting of long-term memory traces through modulation of the EEF-2 elongation factor and suggesting a novel regulatory mechanism linking control of protein elongation, actin cytoskeleton structures, synapse remodelling and forgetting.

776C Identifying novel interactors that regulate *lect-2*/LECT2 function during neurodevelopment

Ian Stone[1], Ashtyn Zoli[2], Marie Kane[2], Maria Lazaro-Pena[2], Carlos Diaz-Balzac[21]Endocrinology, Diabetes and Metabolism, University of Rochester Medical Center, [2]University of Rochester

LECT-2/LECT2 is a secreted protein associated with antimicrobial immunity, inflammatory diseases, amyloidosis and neurite branching. It is conserved through evolution from nematodes to humans, but its function within the nervous system is still not completely understood. LECT-2 works as a cell non-autonomous regulator of dendrite branching of the PVD mechanosensory neurons in *Caenorhabditis elegans* (*C. elegans*). It accomplishes this through its interaction with the menorin complex, including the cell surface receptors SAX-7, MNR-1 and DMA-1. Surprisingly, we observed that LECT-2 is constitutively expressed throughout the lifespan of the animal and its expression was not restricted to areas important for PVD dendrite branching. It was also observed to localize to several neuronal structures, including the nerve ring, motor neurons and other neuronal processes throughout the body. These observations suggest that LECT-2 has other functions in the *C. elegans* nervous system aside from dendrite branching. However, the importance of this striking localization pattern and how it is regulated at the molecular level has not yet been determined.

We have pursued a forward genetic approach using an endogenous, fluorescently tagged reporter of LECT-2 to identify mutants that (1) are deficient in LECT-2 expression/secretion or (2) have abnormal LECT-2 localization. We have previously shown that LECT-2 localization is dependent on SAX-7/L1CAM, although it doesn't affect its expression or secretion. However, mutants of other members of the menorin complex, such as MNR-1 or DMA-1, did not affect LECT-2 localization. In a small pilot screen of 700 genomes, we isolated 2 alleles of SAX-7 and 1 novel mutant that had abnormal LECT-2 localization. We will report on the progress of characterizing the newly identified mutations and their effect on *lect-2*/LECT2 biology. Characterization of the identified mutants will provide us with a better understanding of the role of non-autonomous signals from the muscle, such as *lect*-2/LECT-2, during the development of the nervous system.

777C A Role for Activity Dependent Alternative Splicing in Regulating Neuronal and Behavioral Plasticity

Jamie Stonemetz[1], Nathan Harris[1], Mike Zoberman[2], John Calarco[2], Piali Sengupta[11]Brandeis University, [2]University of Toronto

Alternative splicing (AS) in response to neuronal stimulation has been reported to occur via multiple mechanisms and plays multiple roles, including fine-tuning synaptic plasticity and controlling nuclear export of transcripts. There is, however, limited research connecting activity dependent AS events to behavioral outcomes. The AFD neurons sense temperature and exhibit a form of experience-dependent plasticity, such that the recently experienced temperature influences the temperature at which the sensory response in AFD is activated and the corresponding synaptic output. This response and the corresponding behavioral plasticity are, in part, mediated by changes in gene expression that occur in response to temperature(Yu et al 2014). Previous work in the lab has used translating ribosome affinity purification (TRAP) to identify genes that exhibit differential isoform usage in response to temperature change, suggesting that activity regulated AS events may contribute to neuronal plasticity. To further investigate the regulation and consequences of these AS events, we selected candidate genes for which to generate in-vivo two-color AS reporters. Of the 8 candidate genes that we designed reporters for, 6 of them validated the TRAP data and demonstrated temperature-dependent alternative splicing and 4 of those demonstrated AFD-specific temperature-dependent alternative splicing. One of these candidate genes, *atf-7*, is a bZIP transcription factor and CREB5 ortholog. Using the two-color reporter, we confirmed that *atf-7* undergoes a 3' AS site event in response to changes in temperature. We also found that this AS event is specific to AFD, with other cells expressing consistent ratios of isoforms regardless of changes in temperature. We also examined the functional relevance of *atf-7*. Both cell-specific and whole animal deletion of *atf-7* results in improved negative thermotaxis on a relatively steep gradient. We are currently assessing the contribution of specific *atf-7*isoforms to AFD-driven behavior, as well as examining the upstream regulatory mechanisms of this AS event. Our results suggest that activity-dependent AS events in individual neurons may contribute to experience-dependent behavioral plasticity.

778C Using *C. elegans* to study a promising therapeutic target for Chemotherapy-Induced Peripheral Neuropathy (CIPN)

Lexy Strom[1], Chandni Jaisinghani[2], Hongfei Ji[2], Manuel Axel Ruiz[2], Miriam B. Goodman[21]Biophysics, Stanford University, [2]Molecular & Cellular Physiology, Stanford University

Life-saving cancer chemotherapy impairs touch sensation and gait in tens of millions of cancer patients. These neuropathic and toxic effects are collectively termed chemotherapy-induced peripheral neuropathy (CIPN). There is no FDA-approved treatment for CIPN despite its widespread occurrence and association with increased healthcare costs (PMID: 22482054). Two first-line, microtubule-targeting chemotherapies, vincristine (VIN) and paclitaxel (PTX), frequently result in CIPN. Prior *C. elegans* research links microtubule destabilization through the loss of *pmk-3* to deficits in touch sensation (PMID: 21368137), providing a key entry point for CIPN therapies using the conserved human homolog p38 Mitogen-Activated Protein Kinase (MAPK), for which the pharmaceutical industry has developed dozens of small-molecule inhibitors. Using classical touch assays and high-throughput motility assays in *C. elegans*, we found that VIN and PTX impair touch sensation and movement, mirroring CIPN symptoms. Chemotherapy-induced deficits are suppressed in *pmk-3* (p38-MAPK) mutants but not in mutations of other upstream or downstream MAPK signaling genes, including *tir-1, dlk-1, mak-2,* and *cebp-1*, providing genetic proof-of-concept that p38-MAPK is a plausible therapeutic target. Current work aims to determine whether the protective effect of *pmk-3* loss-of-function results from changes in binding partners or kinase activity.

779C Investigating the protective effects of vitamin B12 on amyloid-beta proteotoxicity

Karli Paige Sunnergren, Caden MacHenry, Rachel Wang, McKenna Millar, Jessica Tanis Biological Sciences, University of Delaware

Alzheimer's disease (AD) is a progressive, multifactorial neurodegenerative disorder that is the leading cause of dementia. Pathological features of AD include accumulation of amyloid-beta (Aβ) and hyperphosphorylated tau, bioenergetic defects, altered mitochondrial morphology, and oxidative stress. By addressing modifiable risk factors such as diet, up to 40% of dementia causes could be delayed or prevented entirely. Contrary to the complex diet of mammals, *C. elegans* eat a simple diet of *E. coli*. Transgenic expression of toxic human Aβ1-42 in *C. elegans* body-wall muscles results in time-dependent paralysis, reduced ATP, defects in mitochondrial morphology, and oxidative stress. We previously discovered that feeding animals with muscle-expressed Aβ a diet rich in vitamin B12 delays the onset of paralysis and alleviates mitochondrial fragmentation, bioenergetic defects, and oxidative stress. The protective effect of vitamin B12 requires methionine synthase, indicating that B12 has this impact by functioning as an enzyme cofactor, rather than as an antioxidant. To assess the impact of vitamin B12 on the nervous system, we transitioned to using *C. elegans* that express pan-neuronal Aβ1-42. As day-five adults, neuronal Aβ animals have a reduced chemotaxis index (CI) to the chemoattractant isoamyl alcohol (IA) when fed a B12-deficient diet, but a wild-type CI when given a B12-rich diet. To determine if neuronal Aβ affects learning, worms were conditioned to IA in the absence of food before the chemotaxis assay. Conditioned control worms associate IA with starvation, but neuronal Aβ worms fed a B12-deficient diet exhibit learning defects. Dietary supplementation with B12 restores learning in neuronal Aβ worms. To determine if these learning deficits are due to defects in the morphology of the AWC neurons that sense IA, we performed imaging on day-five adult Aβ and control worms that express RFP in the AWB and AWC neurons. Scoring the dendrites for waviness revealed dendritic defects in the Aβ animals fed a B12-deficient diet, but normal morphology in the dendrites of Aβ animals fed a B12-rich diet. These results suggest that vitamin B12 supplementation can prevent Aβ-induced defects in neuron function and dendrite morphology.

780C Whole-brain dynamics underlying persistent response to electric shock

Ryoga Suzuki, Kotaro Kimura Grad Sch Sci, Nagoya City Univ

The response of an animal's nervous system in general is temporally associated with external stimulus—for example, a transient stimulus causes a transient neural response. However, in certain cases, a persistent response to a transient stimulus is observed, which is considered to play a critical role in higher brain functions, such as working memory, decision-making, and emotion. Despite its potential significance, the mechanism generating persistent neural activity remains elusive.

We have recently reported that *C. elegans* exhibits a persistent increase in locomotory speed lasting 1-2 minutes after receiving a 30-s AC electrical stimulus (Tee et al., Genetics 2023). Genetic analysis revealed that the persistent speed increase extends to several minutes in *egl-3* loss-of-function mutations, indicating that neuropeptide signaling down-regulates the duration of persistent response to transient stimuli (unpublished observation). However, the neural basis of this persistent response has not been revealed.

In this study, we measured whole-brain activity to an electric stimulus in 54 individuals and identified approximately 9,000 neuronal responses using our 3D microscope system for calcium imaging and NeuroPAL (Wen et al., 2021; Yemini et al., 2021). We found that a five-second electrical stimulus evoked both transient and persistent responses. Transient responses were primarily observed in sensory neurons, while persistent responses lasting several minutes were found in several interneurons, including premotor interneurons. These findings suggest that the transient sensory responses are transformed into persistent activity during information flow in the network. Interestingly, multiple regression analysis suggests that the transient responses in specific sensory neurons affect the duration of persistent interneuron activity. We are currently analyzing the neurons and circuits responsible for generating persistent activity from transient stimulus.

781C Transcriptomic profiling of ASE neurons during associative learning

Armand Olteanu, Leo T. H. Tang Biology, University of Vermont

The remarkable associative learning ability of *C. elegans* with different salt concentrations makes it an attractive model to investigate the cellular and molecular mechanisms of learning and memory encoding. Animals that have been conditioned to a higher salt concentration in the presence of food for twelve hours prefer the high salt region during a salt preference assay. We have previously shown that this is accompanied by a change in synaptic connectivity between the salt sensing ASE neurons and the gustatory AWC neurons. This connection is left-biased in naïve animals but becomes right-biased in high salt conditioned animals. Upon further characterization we uncovered that this switch in connectivity is transcription and translation dependent. We also demonstrated that this connectivity is regulated by the insulin-responsive transcription factor DAF-16/FOXO. The ASE to AWC connection loses its asymmetry when a dominant negative phosphor-null mutant of DAF-16/FOXO is overexpressed in ASE neurons. Therefore, transcriptional control is key to regulating the connectivity between these neurons, and as a consequent, the salt preference of the animal. To find out precisely what genes are being regulated to modulate synaptic connectivity, we are performing ASER and ASEL specific transcriptomic profiling through RNAseq after FACS. With this approach, we can identify differentially regulated genes between naïve and conditioned animals, and between ASER and ASEL. These genes could be responsible in directly modulating synapse formation and elimination, and thus allowing us to understand the molecular mechanisms underlying connectivity-modulated learning.

782C The role of early-life stress on post-embryonic neuronal maturation in *Caenorhabditis elegans*

Ananya Theneti[1], Gabrielle Prince[2], HaoSheng Sun[21]University of Alabama at Birmingham, [2]UAB Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham

Extrinsic factors such as environmental stressors heavily influence post-embryonic nervous system maturation. Prior studies have shown that early life stress (ELS) can increase the incidence of neurodevelopmental and psychiatric disorders. ELS has also been linked to the modulation of various neural circuitry, including the dopaminergic and serotonergic pathways. However, the exact mechanism by which ELS and other extrinsic environmental factors influence post-embryonic neuronal development remains poorly understood. Using the *C. elegans* model, we investigated the role of ELS in the form of L1 starvation on post-embryonic nervous system maturation. Using an automated worm tracking system, we examined locomotion to assess functional neuronal maturation after acute (24 hours) and prolonged (96 hours) L1 starvation stress. We found that prolonged, but not acute, ELS impaired neuronal maturation, evidenced by reduced speed and forward locomotion in adult animals. Concurrently, we examined how dopamine and serotonin – neurotransmitters with conserved pathways in worms and humans – modulate the effects of ELS, given their roles in psychiatric and neurodevelopmental disorders, as well as their involvement in locomotion and signaling of food/nutrient availability in *C. elegans*. Future directions for this study will explore other modes of neurotransmission, including neuropeptides, which are highly implicated in anxiety and other neural disorders. Our long-term goal is to establish a paradigm that will allow us to identify conserved mechanisms underlying stress-induced neuronal impairments to better understand neural disorders.

783C Mild stress affects learning across multiple generations that nicotine may influence

Aicha B Tokar Falatah, Meigan Q Butler, Celeste D Gould, Jacqueline K Rose Psychology, Western Washington University

Maternal stress has been implicated in behavioral deficits associated with anxiety, depression, and schizophrenia in rodent models. Work from our lab has shown that mild stress affects later motor responses to eliciting stimuli in the parent, and subsequent generations. We initially hypothesized that exposure to in-utero stress would lead to differences in learning behavior in Caenorhabditis elegans (C. elegans) across multiple generations. To test this, we used a mild mechanical stressor (liquid suspension on a shaker for 4 hours) and assessed associative learning across four generations (PO-F3). Associative learning was examined by delivering repeated pairings of a mechanical vibration and a blue light stimulus that drive opposing motor responses. After conditioning, worms typically show a change in motor response to the single test stimulus, vibration alone. Our findings reveal that in-utero stress affected learning, seen as an alteration in the stimulus-induced motor response during presentation of a single test stimulus, in the PO generation. As well, initial trials indicate that learning behavior was also affected across generations, with intergenerational effects observed in PO-F2 and transgenerational effects emerging in F3. To explore if nicotine exposure could mediate these effects, worms were exposed to nicotine for two hours before conditioning trials. Understanding the mechanisms that regulate behavior across generations could offer deeper insight into the epigenetic effects of stress on learning and the conditions that could further impact these effects.

784C Investigating mechanisms of NR4A-type nuclear hormone receptors in nervous system development

Candace Tong-Li[1], Niels Ringstad[21]MD-PhD, Neuroscience, NYU Grossman School of Medicine, [2]Neuroscience, NYU Grossman School of Medicine

During nervous system development, an impressive diversity of neuronal subtypes with distinct functions and morphologies are generated. Mechanisms that couple neural activity to gene expression allow environment and experience to further modify neuronal fates after development. Understanding the molecular pathways that establish and modulate the gene regulatory programs that define specific neuronal fates remains a central question in neuroscience. One class of transcription factors - the NR4A-type nuclear hormone receptors - is important for the establishment and maintenance of specific neuronal fates in vertebrates. Despite their importance in neurodevelopment and their link to neurological disease, little is known about the biology of these transcription factors, including how their activity is regulated *in vivo* and how they regulate transcription of specific gene targets. We recently found that the *C. elegans* NR4A homolog NHR-6 is required for the function of chemosensory BAG neurons, offering the opportunity to use these neurons as a model to determine how NR4A-type factors function in neurodevelopment. We have acquired an allele of *nhr-6* that tags the endogenous protein with GFP and includes a degron tag that permits targeted depletion of NHR-6 protein from specific cells and tissues. Using this allele, we will determine (1) whether NHR-6 function is required during development, post-developmentally, or both, and (2) how loss of NHR-6 affects transcription in BAG neurons. We will analyze the NHR-6-regulated genes for sequence features that might identify cofactors which work with NHR-6 to regulate transcription. We hope these studies will advance understanding of NR4A-type nuclear hormone receptors, and the role they play in nervous system development.

785C Exploring Olfactory associative learning in *C. elegans* in response to pathogenic bacteria

Tsung-Lin (Gavin) Tsai[1], Alejandro Aballay[2,31]UT MD Anderson Cancer Center, [2]Department of Genetics, UT MD Anderson Cancer Center, [3]Department of Microbiology and Molecular Genetics, McGovern Medical School at UTHealth

Caenorhabditis elegans exhibits the capacity to modify its olfactory preferences upon encountering pathogenic bacteria, thereby adapting its behavior based on environmental cues. While certain bacteria trigger strong aversive responses, others do not, highlighting the complexity of the C. elegans olfactory detection system. Building upon our previous work on the neural circuits involved in pathogen avoidance, we investigated whether C. elegans can learn to associate Salmonella enterica odor with adverse outcomes. Initially, we conducted choice assays to assess the innate attraction of C. elegans to S. enterica odors, confirming that naïve animals are drawn to them. To determine whether exposure to S. enterica odor influences future behavioral responses, we exposed C. elegans to S. enterica odor while feeding them Pseudomonas aeruginosa overnight. The following day, choice assays revealed a diminished attraction to S. enterica odor, indicating that C. elegans had learned to associate the odor with an adverse experience. These results demonstrate that C. elegans is capable of pathogen-specific olfactory associative learning, providing insights into the mechanisms by which environmental cues influence behavior. Future studies will focus on elucidating the S. enterica molecular cues and C. elegans neural pathways underlying this learned response.

786C The effect of novel bacterial metabolites on egg-laying behavior in C. *elegans*

Martina Velasquez Lemaitre[1], Yen-Chih Chen[2], Mohammad R Seyedsayamdost[3], Christopher Fang-Yen[4], Niels Ringstad[51]NYU School of Medicine, [2]Columbia University, [3]Department of Chemistry, Princeton University, [4]Department of Biomedical Engineering, The Ohio State University, [5]Cell Biology, NYU School of Medicine

Microbially synthesized natural products serve as a major source of therapeutic discovery across various fields, including neuroscience. Many of these therapeutics target the serotonergic circuit, which regulates key physiological functions and behaviors, such as sleep, appetite, learning, memory, and motor function. In *Caenorhabditis elegans*, serotonin agonists induce egg-laying behavior, making it an ideal model for studying serotonergic signaling. To identify novel microbial metabolites that promote serotonin signaling, we adopted a behavior-based screening approach. We screened 864 microbial extracts derived from bacteria exposed to panels of elicitors capable of activating expression of dormant biosynthetic operons. We identified three extracts that consistently increased rates of egg-laying across multiple trials. These three extracts are currently being fractionated and analyzed by mass spectrometry with the goal of identifying the active compounds. This study highlights the potential of microbial natural products as modulators of neurochemical signaling pathways.

787C Peptidergic signaling in establishing experience-dependent behavioral states of *C. elegans*

Sandeep Venkatraman, Ellen Geens, Hanne Janssen, Nathan De Fryut, Sara Van Damme, Isabel Beets Biology, KU Leuven

The ability to adapt is essential for animals to navigate dynamic environments. As animals explore their surroundings, they continuously integrate sensory information with their past experiences and internal states to generate adaptive behavioral states that influence sensory perception and motor output. These persistent states are often controlled by neuromodulatory systems, such as neuropeptides. Yet, our understanding of how their concerted actions govern behavioral states is limited.

In *C. elegans* neuropeptidergic signaling regulates behavioral states shaped by various environmental cues, such as oxygen (O2). Prolonged exposure to low O2 levels adapts O2-escape behavior and sculpts responses to other sensory cues, such as carbon dioxide (CO2). The BAG neurons are important players in these behavioral adaptations, as they sense both low O2 and aversive CO2 levels. Here, we examined the role of BAG-derived peptidergic signaling in O2 and CO2 escape behaviors in animals acclimated to either high (21%) or low (7%) O2 environments.

Disruption of BAG peptidergic signaling through cell-specific deletion of the *egl-3* gene, crucial for neuropeptide processing, affects O2 and CO2 escape behaviors in an experience-dependent manner. We find that BAG peptidergic signaling promotes O2 escape, by accelerating forward movement and suppressing turning behavior, specifically in animals grown at low O2. This effect is phenocopied by mutants of the neuropeptide genes *flp-17* and *flp-19*, which are highly expressed in BAG neurons. FLP-17 signaling also suppresses turning in response to an aversive CO2 cue, independent of previous O2 experience. However, disruption of multiple neuropeptides through BAG-specific deletion of *egl-3* results in reduced turning upon CO2 stimulation, suggesting other BAG-derived neuropeptides have compensatory effects. Future work will further dissect the molecular pathways and cellular circuitry governing these experience-dependent behavioral changes. We expect this will provide functional insight into the organization and plasticity of peptide signaling networks regulating behavioral states.

788C Serotonin signaling and *rig-6*/CNTN6 establish a framework allowing for neuron remodeling in *C. elegans* adult males

Sophia M. Villiere, Kristi Zoga, Michael P. Hart University of Pennsylvania

Changes in neuronal and synaptic wiring may underlie neurological variations such as autism, schizophrenia, and major depressive disorder. Developmental axon guidance cues, postsynaptic receptors, and other molecular and signaling mechanisms govern axon and neurite extension required for circuit formation. Plasticity after development can include remodeling of neuron morphology and synapses to modify circuits and change behavior. What signals and molecular mechanisms allow for neurite remodeling after development? I hypothesize that neurite remodeling in adult *C. elegans* employs a mechanistic framework that overlaps with neurite outgrowth in axon regeneration (in adulthood). I will exploit a model of structural plasticity in *C. elegans* (DVB neuron) to investigate axon regeneration genes in post developmental neurite remodeling. We monitored DVB morphology and associated behaviors, including spicule protraction (aldicarb assay), from the last larval stage (L4) to day 5 of adulthood and identified multiple genes (*daf-18, rig-6, vhp-1, dlk-1, tph-1* and *sdn-1*) that contribute to DVB neuron remodeling in adult males. Mutants of the synaptic adhesion molecule, *rig-6*, have reduced DVB neurite length and reduced time to spicule protraction specifically in day 1 adults. *rig-6* is expressed in male DVB during development, but decreases from day 1 to day 3 of adulthood. We are now investigating where *rig-6* functions. Loss of serotonin through *tph-1* mutation increased DVB neurite length and behavior at day 1. Application of serotonin to control males has the opposite impact, and can rescue the phenotypes of *tph-1* mutants. Serotonin receptor, *ser-7*, is expressed in male DVB but not hermaphrodite DVB, and we are testing *ser-7* for impact on DVB morphology and behavior. We will evaluate the temporal functions of serotonin in adulthood remodeling. Our work suggests that axon regeneration genes impact DVB remodeling, either promoting (i.e. *rig-6*) or inhibiting (i.e *tph-1*) DVB plasticity at day 1 of adulthood. This work provides insight into the genes, molecular mechanisms, and temporal regulation of GABAergic neuronal and behavioral plasticity, suggesting temporal mechanistic frameworks that permit and promote neurite outgrowth after development.

789C Cell fate maintenance of the touch receptor neurons of *C. elegans*

Manon Vleeming[1], Ananya Natarajan[2], Jeroen van Zon[2], Gert Jansen[1]1Developmental Biology, Erasmus MC, [2]Autonomous Matter, AMOLF

The mechanisms governing long-term cell fate maintenance are poorly understood. The genetic networks underlying cell fate determination often contain positive feedback loops that suggest that they act as bistable switches. While such bistable switches explain rapid induction and long-term maintenance of cell fate decisions, they remain inherently reversible, implying that sufficiently strong perturbations or fluctuations could cause spontaneous loss of cell fate. This raises the question whether additional mechanisms exist to make such switches essentially irreversible after induction.

We recently demonstrated loss of ASE neuron fate following transient auxin-mediated depletion of the master regulator CHE-1, demonstrating its reversibility. We also identified a mechanism, target reservoir buffering (TRB), that prevents spontaneous loss of ASE fate, despite its inherent reversibility. In this mechanism fluctuations in CHE-1 level are buffered by preferential binding of CHE-1 to the *che-1* promoter compared to the promoters of CHE-1 target genes.

To examine if reversibility of cell fate and TRB-like mechanism are more widespread, we study touch receptor neuron (TRN) fate maintenance. TRN fate is determined and maintained by a terminal selector transcription factor heterodimer formed by UNC-86 and MEC-3. This heterodimer positively regulates the expression of *mec-3* and binds to a number of target genes required for the function of the TRN cells. *mec-3* expression is stabilized by the transcription factor ALR-1. We use transient auxin-mediated depletion of UNC-86, MEC-3 and ALR-1 to probe reversibility of TRN fate, as measured by recovery of protein levels and touch sensitivity after depletion was stopped.

We found that 24 hrs of auxin-mediated UNC-86 depletion resulted in loss of touch sensitivity. However, after 24 or 48 hrs on plates without auxin, we did not see recovery of the behavioral response, whereas we did observe recovery of UNC-86 protein levels. MEC-3 depletion also caused loss of touch sensitivity, which did not recover after 24 or 48 hrs on plates without auxin. ALR-1 depletion decreased touch sensitivity, but here touch sensitivity did recover after 24 or 48 hrs on plates without auxin. Overall, these results suggest TRN fate is even more readily reversible than ASE fate. We will use simulations, protein depletion and quantitative imaging of protein and RNA levels to elucidate how TRN fate loss is prevented under normal conditions.

790C Investigating the effects of blast-Induced mild-traumatic brain injury on *C. elegans* survival and associative learning

Briannamarie V Wallace, Rohan Gandhi Surapaneni, Timothy A Crombie Biomedical Engineering and Sciences, Florida Institute of Technology

Mild traumatic brain injury (mTBI) is a form of brain trauma that develops from sudden external forces leading to significant cognitive impairments and disruptions within neuronal communication. mTBI can result in cognitive, emotional, and physical symptoms such as memory lapses, attention deficits, and longer-term neurodegenerative risks. *Caenorhabditis elegans*, a small transparent free-living nematode, is a useful model for investigating the neural and molecular bases of mTBI onset and progression. With a simple neural system, well-characterized behaviors, and a wealth of genetic tools, *C. elegans* can be used to study the genes involved in mTBI-associated changes in learning, memory, behavior, and neurodegeneration. Here, we used a custom-built impactor device to deliver repetitive sub-lethal blast exposures to *C. elegans* populations. We then measured the effect of blast exposure on survival and associative learning. We found that repetitive blast exposures of 70 psi did not have a significant effect on survival or associative learning capability. Future work will determine whether stronger blast exposures can modulate learning. By determining a threshold for blast-induced cognitive impairments in *C. elegans*, subsequent research can be done to elucidate the molecular mechanism underlying the progression of mTBI. Ultimately, this work can add to our understanding of mTBI by detailing the progression of cognitive defects in an extremely tractable animal model.

791C Arrestin regulates membrane abundance and function of TMC channels

Rui Wang[1,2], Zhiqiang YAN[2,3], Chaogu ZHENG[1]1School of Biological Sciences, Hongkong University, cgzheng@hku.hk, [2]Institute of Molecular Physiology, Shenzhen Bay Laboratory, [3]Institute for Medical Physiology, Chinese Institutes for Medical Research, zqyan@cimrbj.ac.cn

TMC channels are essential components of the auditory transduction complex in the inner hair cells of the ear. Previous research has shown that vertebrates TMC1 and TMC2 (TMC1/2) function as mechanosensitive ion channels, converting mechanical stimuli into electrical signals in hair cells. However, the difficulties of TMC proteins to localize onto the plasma membrane in heterologous systems and scarcity of the naïve TMC1/2-expressing hair cells hinder the further research of TMC1/2. *C. elegans* has two TMC homologs (CeTMC-1 and CeTMC-2), which are expressed in many tissues, including muscles and hair cell-like ciliated neurons. *C. elegans* TMC proteins have not only mechanosensory functions similar to the mammalian TMC channels but can also serve as alkaline sensors and ion channels that regulate background conductance. A recent structural study discovered a functionally uncharacterized arrestin protein called ARRD-6 that is co-purified with CeTMC-1. Arrestins are known for their roles in negatively regulating G protein-coupled receptor (GPCR) signaling by blocking the coupling between GPCRs and G proteins, but their role in regulating mechanosensitive channels is unknown. We found that the *C. elegans* ARRD-6 regulated the steady-state abundance of TMC-1/2 proteins, suppressed the egg-laying defects in *tmc-1/2* mutants, and was also involved in the habituation of alkaline sensation. Through a set of biochemical experiments, we further found that ARRD-6 likely regulates TMC-1/2 channel abundance and function through the ubiquitination and endocytosis system. Furthermore, the mammalian homolog of ARRD-6 share similar interaction with TMC complex, ubiquitination, and endocytosis system, suggesting a conservative role in regulating and modulating TMC complex. Thus, we propose that arrestins could regulate TMC complex in a way analogous to their regulation of GPCRs, , offering new insights into auditory signal transduction and laying the groundwork for developing preventive and therapeutic strategies for hearing loss.

792C Identification of Sensory Cues that Evoke Contact Response in Mating Behaviors of Male *Caenorhabditis elegans*

Chieh Wang, Jen-Wei Weng, Yi-Shin Wang, Chun-Hao Chen National Taiwan University

Mating ensures genetic diversity throughout evolution, making mate recognition crucial for reproductive success. This process requires animals to identify suitable mates and execute mating behaviors. Our previous research demonstrated that *C. elegans* males respond to cuticular cues from mates by pressing their tails against potential partners, initiating a contact response followed by body circulation until reaching the vulva. This sustained body searching depends on a specific range of body stiffness, leading to persistent changes in locomotor patterns for mate-searching. These findings suggest that *C. elegans* males rely on a two-step sensory mechanism for contact-mediated mate recognition, though the identity of the cuticular cues remains unknown. To investigate the genetic basis of these cuticular cues, we performed an ethyl methanesulfonate (EMS) forward genetic screen and identified a recessive *chc1* mutation in hermaphrodites that significantly reduced male contact responses. Scanning electron microscopy (SEM) analysis revealed that *chc1* mutants exhibited structural defects in cuticular microstructures, including the annulus and alae, highlighting its role in maintaining cuticle integrity. Consistently, *chc1* mutants showed increased cuticle permeability and heightened susceptibility to DiI and Hoechst staining, indicating fundamental molecular-level changes. Notably, while purified cuticles from wild-type hermaphrodites efficiently triggered male contact responses, *chc1* mutant cuticles failed to elicit similar behaviors, suggesting that *chc1* encodes a gene essential for delivering sensory cues to males. To further define the genetic basis of these defects, we conducted single-nucleotide polymorphism (SNP) mapping and whole-genome sequencing to identify the underlying nucleotide changes. Our findings provide novel insights into the genetic framework governing cuticle composition in mate recognition. Given the evolutionary conservation of these mechanisms among nematodes, this research may also contribute to understanding mate recognition in parasitic nematodes.

793C The loss of a chloride channel in glia extends lifespan via pH regulation and activation of multiple protective programs in *C. elegans*

Lei Wang[1,1], Enrique Ismael Oliver Santiago[2], Dario Lorenzo D'Urso[2], Bianca Graziano[2], Laura Bianchi[21]University of Miami, [2]university of Miami

Exciting new data in the model organism *C. elegans* support the notion that glia are involved in organismal aging. The molecular underpinnings of glial control of aging though are for the most part unknown. We recently discovered that knockout of *clh-1*, a gene that encodes a chloride/bicarbonate channel in glial cells, extends lifespan in *C. elegans*. Loss of *clh-1* in glia also reduces accumulation of reactive oxygen species, enhances oxidative stress resistance, and regulates protein homeostasis throughout the organism via activation of autophagy. Our previous research indicated that the loss of *clh-1* results in an increased pH within the glial cells, raising the intriguing possibility that glial pH might regulate stress resistance and aging. To test this hypothesis, we determined the effects of manipulating the expression of the carbonic anhydrase, *cah-4*, in glial cells—either through knockdown or overexpression— on pH, oxidative stress, lifespan, and healthspan. We found a connection between pH alterations in glial cells and the regulation of oxidative stress resistance, healthspan, and aging. Our findings identify a clear relationship between pH regulation in glia and aging, potentially offering insights for the development of anti-aging therapeutics.

794C Connecting mechanosensation and social behavior in relation to ASD risk genes

Lisa Wang[1], Brooke Frohock[2], Swetha Iyer[1], Kaelin Rubenzer[3], Jonathan Pierce[11]Neuroscience, University of Texas in Austin, [2]University of Oregon, [3]University of Texas in Austin

Tactile sensation plays important roles in human social interactions, often in the form of social distance awareness and touch-based communication such as hugs. Mice studies showed that a lack of tactile stimulus during development is associated with atypical social behaviors. This association is observed in human subjects with autism spectrum disorder (ASD) as well. ASD is a hereditary condition, in which more than 90% of the patients exhibit both abnormal tactile responses and social deficits. These observations led us to hypothesize that in ASD patients, there is a causal relationship between abnormal tactile sensation and social deficits at a genetic level. An understanding of this connection could encourage peripheral nervous system-based ASD treatment. This type of treatment would be invaluable, as many ASD medications have off-target effects on the central nervous system.

To investigate the connection between tactile and social deficits in ASD, we examined a genetic-wide association study from the Simons Foundation. This study revealed more than 100 genes that potentially contribute to ASD symptoms. These genes are known as ASD risk genes and their contribution to tactile and social defects remains unclear. We need a high-throughput approach to screen these genes for their association with atypical tactile and social functions. We picked C.elegans as a model for this project because 70% of these ASD risk genes are conserved in C.elegans. Human mechanosensory pathways are also conserved in worms. We utilized a pheromone-guided aggregation behavior in C.elegans named clumping to measure changes in social behaviors in worms. We found several ASD risk gene orthologs that alter clumping behavior. Recently, we identified an additional social behavior named rubbing, which occurs when two worms cross paths and proceed to rub against each other. Genetic mutations that disrupt gentle touch neuron operations alter the occurrence and duration of rubbing in worms. We will leverage rubbing to evaluate the subtle effects of ASD risk genes on social behaviors and mechanosensory neurons, which will help us understand the connection between touch and social functions on a cellular level. To this end, we developed a worm collision detection system that analyzes videos of worm interactions in a time-efficient and unbiased manner. We will utilize this system to identify ASD genes that alter rubbing behaviors and the neuronal function of mechanosensory neurons.

With this approach, we aim to uncover the roles of ASD risk genes in connecting tactile and social functions. A better understanding of this topic could lead to early diagnosis and treatments that improve the life quality of people with ASD.

795C Elucidating the drivers of sex-specific neuronal gene expression in *Caenorhabditis elegans* sensory neurons

Zachary C Ward[1], Carlos A Diaz-Balzac[2], Kelli A Fagan[2,3], Douglas S Portman[21]Biomedical Genetics, University of Rochester Medical Center, [2]University of Rochester Medical Center, [3]University of Rochester

Sexual dimorphism in the nervous system provides a powerful lens for understanding how genetic programs can diversify neural circuit structure and function. In C. elegans, sex differences in chemosensory and locomotor behaviors arise in part from sex-specific patterns of gene expression in sex-shared neurons. This process is controlled by the master regulator gene *tra-1*, which is necessary and sufficient to shape sex-specific neural and behavioral traits. Yet, the effectors of *tra-1* that function in sex-shared neurons that mediate sexually dimorphic behaviors are poorly understood.

Previous work demonstrated that the sensory neuron ADF mediates male attraction to the hermaphrodite sex pheromone ascr#3. The male-specificity of this attraction arises from sex-specific sensory tuning: only genetically male ADF neurons exhibit robust responses to ascaroside stimulation. Using an RNAseq-based approach, we identified *srd-59*, a chemoreceptor expressed exclusively in the ADF neurons of adult males. Through ADF-specific manipulation of the sex determination pathway, we find that *tra-1* acts cell-autonomously in ADF to regulate *srd-59* expression. Surprisingly, *srd-59* expression does not require *mab-3*, a DMRT-family transcription factor known to be a direct target of *tra-1* in ADF. This suggests the existence of unidentified effectors of genetic sex in ADF.

To uncover these regulators, we conducted a forward genetic screen to identify mutants that lack *srd-59* expression in male ADF ("OFF mutants") or ectopically express *srd-59* in hermaphrodite ADF ("ON mutants"). We identified a number of mutants that alter *srd-59* expression but do not disrupt overt sexual differentiation of the soma. We evaluated the specificity of mutant phenotypes by examining the presence of selected male-specific neurons (CEMs and RnBs). Among the OFF mutants, we are focusing on those in which expression of the non-sex-specific ADF marker *srh-142* is not disrupted. We are currently working to identify the causal genetic changes in these mutants. We expect our findings to reveal new insights into the mechanisms by which genetic sex shapes sensory behavior by regulating gene expression in sex-shared neurons.

796C How do social cues modulate intergenerational signaling?

Jadiel Wasson New York University

Classically, inheritance was believed to be restricted to the passage of information from parent to progeny in the form of genetically encoded material. It has become appreciated that other types of information, including that which informs about the environment, can be passed between generations. However, the mechanisms behind how this information can be both passed on and interpreted by the embryo remain unknown. Recently, we have identified a previously unknown pathway for intergenerational communication that links neuronal responses to maternal provisioning in *C. elegans.* Here, a chemosensory signaling pathway responsive to social cues initiated in the mother alters the pool of maternally provided factors that modulates gene silencing in progeny. This intergenerational signal transmission depends on specific chemosensory neurons and neuronal FMRFamide (Phe-Met-Arg-Phe)–like peptides including FLP-21. Parental FLP-21 signaling dampens oxidative stress resistance and promotes the deposition of mRNAs for translational components in progeny, which, in turn, reduces gene silencing. Furthermore, overexpression of FLP-21 has the opposing effect on gene silencing in progeny suggesting that the levels of FLP-21 signaling in mothers *influences* embryonic stress phenotypes. Taken together, we hypothesize that loss of social cues in the parental environment represents an adverse environment that stimulates stress responses across generations. This work demonstrates how alterations to chemosensory signaling pathways can have long range consequences via changes in not only what mothers provide to their young, but also how resulting progeny modulate their gene expression changes in response to a challenge. Ultimately, this work will lead to a clearer understanding of the mechanisms involved in cross-generational signaling between mother and progeny.

797C PXF-1 functions in synaptic vesicle cycling through Rap GTPase signaling

Julie Watkins, Martin Werner, Salvatore Cherra Neuroscience, University of Kentucky College of Medicine

Small GTPases play essential roles in synapse development and function. We previously found that mutations in *pxf-1*, a Rap guanine exchange factor, cause neuromuscular dysfunction and reduce the accumulation of synaptic vesicles at the neuromuscular junction. To investigate the underlying mechanisms, we first assessed synaptic bouton size using fluorescence microscopy with freely diffusible GFP or mCherry expressed in cholinergic motor neurons. We observed no differences in bouton size between wild type and *pxf-1* mutant animals. We next examined synaptic vesicle precursor trafficking using SNB-1::GFP but found no significant differences in vesicle movement or directionality. These findings suggest that *pxf-1* does not alter synaptic bouton morphology or vesicle precursor trafficking. Given the reduced accumulation of synaptic vesicles in *pxf-1* mutants, we hypothesize that PXF-1 may regulate synaptic vesicle dynamics at the level of exocytosis and/or endocytosis. To test this, we are assessing interactions between *pxf-1* and factors involved in vesicle cycling. *unc-41* encodes an endocytic factor required for synaptic vesicle component retrieval, and *unc-64* encodes a component of the exocytic SNARE complex. We measured the intensity of mCherry::RAB-3 in *pxf-1* mutants in the presence or absence of *unc-41* and *unc-64* mutations to determine whether PXF-1 influences vesicle recycling or fusion efficiency. These experiments will clarify the role of PXF-1 in synaptic vesicle dynamics and its potential regulation via Rap GTPase signaling.

798C Coelomocytes display immune-like activation by mobilizing and ramifying with alcohol

Chelsea Webber, Ben Clites, Zheng Wu, E. A. Cardona, Jon Pierce Neuroscience, The University of Texas at Austin

Consumption of alcohol causes intoxication. Aside from physiological changes in the nervous system, alcohol causes dramatic activation of immune cells. At both morphological and transcriptional levels, alcohol causes macrophages in the periphery and microglia in the brain to increase in number, increase expression of proinflammatory genes, and retract their processes to assume an ameboid shape. This dramatic immune response has driven researchers to study how and why alcohol activates immune cells.

For two decades, *C. elegans* has been used to elucidate molecular bases for behavioral response to alcohol that are conserved in humans. This has included ion channels and neuropeptide pathways, but as of yet, no connection with immune signaling.

To search for novel genetic modifiers of intoxication, we performed a genome wide association for intoxication in *C. elegans*. This pointed to a deleterious variant in the putative nicotinic acetylcholine receptor, *lgc-24*, which may cause hypersensitivity to alcohol. We confirmed a role for *lgc-24* in alcohol responses with several models of intoxication in worm. First, deletion of *lgc-24* in N2 caused hypersensitivity to suppression of egg laying which was rescued with *lgc-24(+).* Second, using a swimming model of intoxication, we found that a wild isolate with a deleterious variant in *lgc-24* was hypersensitive - slowing and spending significantly more time in a curled position with alcohol treatment.

The *lgc-24* receptor is almost exclusively expressed in coelomocytes, which is a cell type that resembles macrophages and microglia in its endocytic functionality but has not been known to activate morphologically. Coelomocytes typically exist in three ovoid pairs distributed evenly along the body. We found that treatment with alcohol caused a dose-dependent activation of the anterior coelomocyte pair in N2 background. Coelomocyte activation was apparent as they unpair, passively mobilize, and present with ramifying processes. Intriguingly, we also found that wild strains carrying deleterious variants in *lgc-24* have chronically activated coelomocytes even in untreated worms.

We conclude that a) *lgc-24* has a critical role in coelomocyte homoeostasis and disruption of this receptor leads to cell activation, and b) coelomocytes activate morphologically in response to ethanol exposure suggesting a previously unknown immune function. This research may further our understanding of acetylcholine in ethanol-mediated inflammation.

799C Deciphering motor neuron vulnerability in *C9ORF72* ALS by developing novel *C. elegans* models

Ian Q Weigle, Jessica S Markman, Yihan Chen, Paschalis Kratsios Neurobiology, University of Chicago

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease without effective treatments. ALS primarily leads to the progressive loss of motor neurons (MNs) and paralysis. The most common genetic cause of ALS is an unusual mutation - a GGGGCC (G4C2) hexanucleotide repeat expansion in the first intron of *C9ORF72*. Although loss of C9ORF72 protein expression has been suggested, strong evidence across model systems indicates that the G4C2 repeat mutation primarily contributes to disease through two gain-of-function mechanisms: repeat RNA toxicity and dipeptide repeat (DPR) protein toxicity. However, the molecular mechanisms driving the selective vulnerability of MNs to *C9ORF72* ALS remain poorly understood. To identify such mechanisms, I leverage the specific strengths of *C. elegans*, a genetically tractable system ideally suited for *in vivo* modeling of ALS and unbiased genetic screens. The Kratsios lab has recently developed *C. elegans* models expressing 75 G4C2 repeats either ubiquitously or exclusively in neurons (PMID: 34654821). These two *C. elegans* models recapitulate key ALS features, including DPR aggregation, MN degeneration, locomotor deficits, and reduced lifespan. Further, my preliminary data suggest that certain non-MN populations, such as mechanosensory neurons (ALMs, AVM, PLMs, PVM, PVD) are resistant to G4C2-induced toxicity. We therefore hypothesize that cell type-specific molecular pathways confer differential vulnerability to *C9ORF72*-related neurotoxicity. To this end, we characterize novel cell type-specific strains with G4C2 expression restricted to either cholinergic motor neurons, mechanosensory neurons, or dopaminergic neurons. Our preliminary analyses using these strains reveal differential vulnerability across these neuronal populations, reinforcing the hypothesis that intrinsic molecular pathways modulate neurotoxicity. We also find that G4C2 expression alters neuromuscular junction integrity, as evidenced by loss of presynaptic active zone protein, CLA-1, in cholinergic MN DA9. Finally, I leverage the optical transparency of *C. elegans* to visually track DPR accumulation *in vivo* for genetic screens aimed at identifying conserved factors mediating MN susceptibility and mechanosensory neuron protection. This project will provide crucial insights into the mechanisms underlying MN vulnerability and non-MN protection in *C9ORF72* ALS, providing a platform for uncovering novel therapeutic targets for *C9ORF72* ALS.

800C Microbiome-derived Short Chain Fatty Acids Promote Motivation of Mate Searching via A Gut-to-Brain Signaling through Octopamine in *C. elegans* Males

Jen-Wei Weng[1], Yi-Sin Wang[1], Li-Ren Wang[2], Chun-Hao Chen[11]National Taiwan University, [2]Taipei Municipal Zhongshan Girls High School

Microbiomes modulate host behavior through gut-brain communication, yet the underlying neural mechanisms remain poorly understood. The nematode *Caenorhabditis elegans*, a bacterivorous organism with a well-characterized nervous system, serves as a powerful model for studying host-microbiome interactions. Here, we investigate how bacterial diets influence male mate-searching motivation in *C. elegans*. We find that males exhibit high mate-searching motivation on the standard laboratory diet, *Escherichia coli* OP50, while motivation varies across bacterial strains from the *C. elegans* microbiota (CeMbio). Notably, *Comamonas*, particularly *Comamonas aquatica* DA1877, strongly suppresses mate-searching motivation. Through a transposon screen in DA1877, we identify propionyl-CoA carboxylase (PCC) and acetyl-CoA carboxylase (ACC), two enzymes involved in short-chain fatty acid metabolism, as a key mediator of this effect. However, bacterial genes involved in vitamin B12 synthesis do not contribute to the suppression. Similarly, disrupting *pcca-1* (propionyl-CoA carboxylase) and *acdh-1* (acyl-CoA dehydrogenase) in the host, which leads to propionate accumulation, restores mate-searching behavior in DA1877-fed males, suggesting that propionate plays a central role in regulating sexual motivation. Furthermore, two G-protein-coupled receptors (GPCRs) with restricted neural expression appear to act downstream of propionate, converging signals onto a pair of octopaminergic RIC interneurons. Our findings reveal a metabolic-neuronal pathway through which bacterial metabolites influence male sexual motivation in *C. elegans*. This study provides insights into the complex interplay between microbial metabolism, host neural circuitry, and behavior, expanding our understanding of gut-brain communication.

801C A sensory cilium mediates specific neuron-glia attachment

Leland Wexler[1], Irina Kolotuev[2], Brian Griffin[3], Niels Ringstad[3], Maxwell Heiman[41]Genetics, Boston Childrens Hospital, [2]University of Lausanne, [3]New York University School of Medicine, [4]Harvard Medical School

Glial cells form specialized attachments with specific neuronal partners, but how such precise cell-cell pairings occur remains unknown. Here, we show that sensory cilia mediate the highly stereotyped attachments of two sensory neurons, BAG and URX, to a single partner glial cell, the lateral inner labial socket (ILso). The URX attachment is particularly remarkable as its dendrite is positioned in the dorsal sensory bundle of the head and makes a "jump" across the nose tip to attach to ILso in the lateral sensory bundle. We found that BAG and URX form their attachments to ILso through a multi-step process. First, in comma stage embryos, BAG and URX anchor to guidepost glial cells via the adhesion protein SAX-7 and the scaffolding proteins GRDN-1 and MAGI-1, which causes their dendrites to extend by stretch during embryo elongation. Second, after dendrite extension is complete, BAG and URX form their mature attachments to ILso by extending their sensory cilia. In *daf-19* mutants, in which cilia do not develop, BAG and URX fail to attach to ILso. Using a visual forward genetic screen for loss of cilia attachment, we identified a gene we named *bug*-1 (for BAG/URX-Glia defective) that is required for neuron-glia attachment. In *bug-1* mutants, the BAG and URX cilia are present, but fail to attach to ILso. *bug-1* encodes a predicted >350 kD secreted protein that includes two CASH carbohydrate-binding domains, several EGF domains, and a Cadherin-like domain. It is expressed embryonically in BAG and URX at the time when the cilia-glia attachment forms but is not expressed in ILso glia. Using endogenous deletions, we found that the CASH and EGF domains each promote BAG and URX cilia attachment to ILso, while the Cadherin-like domain is largely dispensable. In the absence of URX cilia attachment to ILso, due to loss of *bug-1* or ILso ablation, URX cilia fail to adopt activity-dependent branching patterns normally seen in adult animals, suggesting a requirement for glia in cilia plasticity. Notably, EM studies recently revealed extensive cilia-mediated attachments among neurons and glia in the human brain. Our work shows that similar contacts are present in *C. elegans* and can be used to define the molecular and functional basis of cilia-mediated neuron-glia attachment.

802C Octopamine neurotransmission and CREB activation confer neuroprotection in a *C. elegans* model of Parkinson's disease

Corey W. Willicott, Guy A. Caldwell, Kim A. Caldwell Biological Sciences, The University of Alabama

Parkinson's disease (PD) is characterized by progressive dopaminergic (DA) neurodegeneration, yet the interplay between DA signaling and neuroprotection remains unclear. Using a *C. elegans* model expressing the human A53T α-synuclein mutation exclusively within the DA neurons, we found that loss of EGL-30 (Gαq) via crossing to *egl-30(n686)* enhanced DA neurodegeneration, whereas GOA-1 (Gαo) [*goa-1(sa734)*] rescued these effects. Additionally, when A53T α-syn worms were crossed to *cat-2* mutant animals, the loss of dopamine itself conferred neuroprotection. Consistent with this, neurodegeneration analysis revealed that CEP neuron ablation was protective for ADE neurons in A53T-overexpressing animals. Previous work showed that dopamine released from CEP neurons binds to the inhibitory receptor, DOP-3 resulting in octopamine release from RIC interneurons. Octopamine subsequently binds to the SER-3 biogenic amine receptor in SIA neurons leading to non-canonical activation of CRH-1 (CREB) expression via CRTC-1, a CREB co-activator. To investigate whether this mechanism applies to DA neurodegeneration, we conducted qPCR analysis of A53T-overexpressing worms and observed a significant increase in *crtc-1* mRNA levels, which was diminished in a hypomorphic allele of *egl-30(n686)*. This suggests that EGL-30 signaling normally activates CRTC-1. In the context of the neuronal connectome map, we hypothesize that the loss of dopamine enables RIC-SIA octopamine-mediated communication, ultimately leading to CRH-1 activation and neuroprotection. Neurodegeneration analysis of *dop-3(vs106)*, *ser-3(ad1774)*, and *tbh-1(n3247)* loss-of-function mutants with A53T and in the *cat-2(n4547)* mutant background will provide further support for this model. Taken together, these findings suggest a novel octopamine-mediated pathway that may contribute to DA neuroprotection and provide insight into potential therapeutic strategies for PD.

803C The *gba-3* gene encodes a glucocerebrosidase that exacerbates α-synuclein mediated impairments in *C. elegans* mutants

Ning Liu[1], Rongzhen Li[1], Xiaobing Huang[2], Merja Lakso[1], Garry Wong[1]1University of Macau, [2]Shenzhen Technology University

Glucocerebrosidases (GCases) are enzymes that catalyze the hydrolysis of β-glucoceremides and β- glucosphingosines to produce glucose and ceramide or sphingosine, respectively. In humans, loss of function mutations in GBA1 encoding a lysosomal GCase, result in accumulation of glucosylsceramide in lysosomes and underlie Gaucher's disease (GD). Interest in GBA1 function resides not only in understanding lysosomal storage diseases but also its link to Parkinson's disease (PD), as specific mutations in GBA1 are the highest known genetic risk factors (odds ratio >5) for PD. Nonetheless, how GCases are linked to PD at the molecular level is limited due to lack of suitable animal models. This study aimed to identify the gene encoding GCase in *C. elegans* through bioinformatics, tests of deletion mutants for enzymatic activity, and transgenesis. Genetic crosses with a nematode PD model followed by metabolomic and transcriptomic analysis was used to characterize the molecular links between GCase and neuropathogenic α-synuclein. We identified *gba-3* as a *C. elegans* GCase with demonstrable enzymatic activity. *Gba-3* loss of function mutants lacked GCase activity. In *gba-3* deletion mutants crossed to human α-synuclein(A53T) overexpressing animals, we observed exacerbation of phenotypic deficits including slowed movement, decreased lysosomal function, accumulation of α-synuclein protein, and impairment of dopaminergic neuron morphology. Lipidomics analysis revealed significant accumulation of glucosylceramides in *gba-3* deletion mutants that was augmented in crosses to α-synuclein overexpressing animals. Gba-3 deletion mutant crosses also had increased α-synuclein protein levels. RNA-seq analysis revealed enrichment of glucosyl transferase genes in *gba-3* mutants and cilia genes in *gba-3* mutant and α-synuclein overexpression crosses. This study shows how loss of *gba-3* function can decrease lysosomal function, increase pathogenic α-synuclein levels, and cause widespread dysregulation of lipids. This study thus provides insight into how GBA1 functional loss is a risk factor for PD. In addition to providing a valuable resource for studies of lysosomal storage and neurodegenerative diseases, this study strengthens and extends knowledge of the relationship of two key proteins in Gaucher's and Parkinson's diseases.

804C Intercellular Sphingolipid signaling mediates aversive learning in *C. elegans*

Yu-Chun Wu[1,2], Isabel Beets[3], Bennett William Fox[4], Diana Fajardo Palomino[4], Li Chen[3], Chien-Po Liao[1,2], Elke Vandewyer[3], Liang-Yi Lin[1,2], Chun-Wei He[1,2], Li-Tzu Chen[1,2], Chih-Ta Lin[1,2], Frank C. Schroeder[4], Chun-Liang Pan[1,21]Institute of Molecular Medicine, College of Medicine, National Taiwan University, [2]Center for Precision Medicine, College of Medicine, National Taiwan University, [3]Animal Physiology and Neurobiology, KU Leuven, [4]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University

Surrounded by diverse species, *C. elegans* learns to avoid potential threats by associative aversive learning. Environmental stressors, such as pathogens, induce mitochondrial stress in *C. elegans*, in particular in the peripheral, non-neural tissues. *C. elegans* then develops avoidance behavior through aversive learning, aiming at reducing the exposure to the stressors. However, the molecules that signal from the peripheral tissues to modulate the neural circuits and enable aversive learning remain unclear. We recently established a laboratory model of aversive learning by associating the food bacteria *E. coli* with mitochondrial stress. We found that sphingosine-1-phosphate (S1P), catalyzed by SPHK-1, plays an important role in stress-induced aversive learning. S1P binds LPR-3, a lipocalin that likely facilitates the extracellular transport of S1P. We further identified the sphingosine-1-phosphate receptor SPHR-1, and revealed its function in the octopaminergic RIC neuron. Importantly, we showed that this S1P signaling pathway was used by *C. elegans* to develop learned aversion for the pathogenic bacteria *Chryseobacterium indologenes*. These findings uncover the molecular pathway by which sphingolipid signaling from peripheral tissues modulates neural circuit functions to promote aversive learning.(supported by the National Science and Technology Council, NSTC 113-2811-B-002-144)

805C Nuclear Hormone Receptor NHR-49 regulates sensory behavior and learning in *C. elegans*

James T. W. Wu[1,2,3], Glafira Ermakova[2,3,4], Lexis D. M. Kepler[5,6], Catharine H. Rankin[5,6], Stefan Taubert[2,3,41]Department of Cellular and Physiological Sciences, University of British Columbia, [2]Centre for Molecular Medicine and Therapeutics, University of British Columbia, [3]BC Children's Hospital Research Institute, [4]Department of Medical Genetics, University of British Columbia, [5]Department of Psychology, University of British Columbia, [6]Djavad Mowafaghian Centre for Brain Health, University of British Columbia

All animals must sense, respond, and adapt to environmental cues to survive. Nuclear Hormone Receptor NHR-49 is a *C. elegans* transcription factor that regulates various developmental and physiological processes, including longevity, stress response, and lipid metabolism. NHR-49 regulates lipid metabolism by regulating fatty acid desaturase genes (*fat-6, fat-7*) and thus maintains normal life span. While neuronal expression of NHR-49 has been shown to promote longevity and stress survival in *C. elegans*, its contributions to behavior and neuron function remains understudied.

In this study, we show that NHR-49 is critical for chemo- and mechanosensory neuron function in *C. elegans*. Animals that lack NHR-49 have defects in chemotaxis to odorants sensed by AWA neurons such as diacetyl and pyrazine. NHR-49 mutants also have intrinsic defects in AWA neuron morphology. Additionally, we found that tissue-specific expression of NHR-49 in the neurons is sufficient to rescue these chemotaxis defects. We also found that NHR-49 mutants have impaired habituation to touch, suggesting that NHR-49 plays a role in the regulation of mechanosensory learning circuits. We are now conducting fatty acid supplementation experiments that restore lipid homeostasis to determine whether the role of NHR-49 in these behaviors may be independent of its canonical role in lipid metabolism. Future experiments aim to identify the genetic pathways through which NHR-49 acts with promote chemo- and mechanosensory behaviors. Our work describes new roles of NHR-49 in sensory behavior and learning and will clarify the role of NHR-49 in neuron function.

806C ASD risk allele increases penetrance and degree of normal individual behavioral trait in *C. elegans*

Grace Wulffraat[1], Swetha Iyer[1], Audrey Brumback[2], Jonathan Pierce[1]1University of Texas at Austin, [2]University of Texas at Austin Dell Medical School

Autism Spectrum Disorder (ASD) is a polygenic condition characterized by a wide array of developmental and behavioral symptoms. As the number of ASD risk genes grows, a major challenge remains to understand how different variants in these genes cause ASD-related phenotypes. This project aims to elucidate cellular and molecular mechanisms underlying ASD.

Because of the large number of genes and variants implicated in ASD, a high-throughput method is crucial for this research. With 75% of ASD risk genes conserved between humans and *C. elegans*, the worm is a powerful model for this study. Researchers have leveraged this system to identify novel ASD risk genes, show phenotypes that result from perturbing these genes, and elucidate pathways involved in ASD at large. Now, we are investigating mechanisms of action for individual variants in ASD risk genes.

To do this, we CRISPR engineer potentially causal mutations from ASD patients in *C. elegans* orthologs of ASD risk genes and identify resulting phenotypes. Through this process, we discovered a novel ASD-associated phenotype. In wild-type *C. elegans* populations, we observe two distinct patterns of swimming behavior. First, a well-characterized 'normal' swimming pattern consisting of repeated alternate dorsoventral 'C'-shaped bends. Second, a novel swimming pattern we named 'conducting' — individuals intersperse dorsoventral C-bends with left and right bends, resembling the hand signals of a conductor. In wild-type N2 populations, we find that 20% of individuals swim with a conducting pattern throughout their lives. Similar low penetrant conducting was observed for undomesticated wild strains. Interestingly, populations of *C. elegans* with a variant in the ortholog of an ASD risk gene, DLG4, display significantly higher proportions of conducting worms. This is intriguing because ASD symptoms have been proposed to represent an increased degree of behavioral phenotypes that are normal, but lowly penetrant in individuals without ASD. Using this novel phenotype, we aim to understand how this variant functions at cellular, synaptic, and systems levels.

Here, we show that allele-specific study of ASD in *C. elegans* has the potential to uncover novel phenotypes, which may advance our mechanistic understanding of ASD. Further, understanding connections between penetrance of behaviors and ASD risk genes in *C. elegans* may provide insight on ASD in humans.

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808C Automated analysis of *C. elegans* behavior by LabGym: an open-source, AI-powered platform

L. Amanda Xu[1], Zhaoyu Li[2], X.Z. Shawn Xu[1], Bing Ye[1], Yujia Hu[1], Elizabeth A. Ronan[1]1University of Michigan, [2]University of Queensland

Despite its simplicity, *C. elegans* exhibits diverse behaviors, including locomotion, swimming, mating, and social interactions. With its genetic tractability and well-mapped circuitry, it is an ideal model for physiological and behavioral studies. However, existing behavior analysis software is often highly specific, non-generalizable, and tedious—requiring extensive coding to analyze each behavioral parameter individually. Most platforms focus on basic locomotor properties, such as body position and speed, which may oversimplify movement dynamics and compromise analytical accuracy. Instead of manually coding every behavioral combination, artificial intelligence offers a promising solution to improve utility and precision. However, no such AI-driven, behavioral analysis platforms have been developed in *C. elegans*.

LabGym—a recently developed, open-source, AI-based behavior analysis software—leverages machine learning to improve the identification, categorization, and quantification of user-defined behaviors across animal species. Here, we aim to introduce *LabGym* to the *C. elegans* research community by developing computational models in *LabGym* for worm behavioral analysis.

Unlike existing platforms that may focus more on simple behavioral parameters, *LabGym* considers spatiotemporal changes and overall motion patterns, providing more holistic and precise assessments of any behaviors of interest. Instead of relying on pre-defined behavioral types, the AI models in *LabGym* are user-customizable, and can recognize and quantify experimenter-defined behaviors with high accuracy and efficiency.

We have trained AI models in *LabGym* to track and analyze acute worm movements including trajectory, forward locomotion, reversals, omega turning, and head-casting with over 90% accuracy. *LabGym* automatically generates quantitative behavioral metrics such as frequency, duration, latency, speed, velocity, acceleration, distance, magnitude, and intensity to quantify identified behaviors. At the same time, it provides built-in statistical analysis and visualization tools for diverse experimental purposes.

We will further develop AI models to analyze additional worm behaviors such as roaming and dwelling, specialized movements such as swimming and undulatory body bends in *unc* mutants, and social behaviors like mating. Beyond these behaviors, *LabGym*'s AI-driven adaptability ensures that it can be customized by the experimenter to characterize any behavior of interest—offering a cost-effective and comprehensive approach to behavioral analysis in *C. elegans*.

809C Roles of *lat-2*/Latrophilin and *ten-1*/Teneurin in Developmental Neurite Pruning in *C. elegans*

Sophia Yevlash, Menghao Lu, Kota Mizumoto Zoology, University of British Columbia

Neurite pruning is a crucial developmental process required for the maturation of functional neural circuits. Defects in this process have been linked to neurodevelopmental disorders, including Autism Spectrum Disorder, ADHD, and schizophrenia (Moreno-Salinas et al., 2019). However, the molecular mechanisms regulating neurite pruning remain largely unknown. We previously showed that the PDB motor neuron undergoes stereotyped neurite pruning (Lu and Mizumoto, 2019). Through a candidate approach, we found that the null mutants of *lat-2*, an orthologue of Latrophilin, result in neurite pruning defects in PDB, indicating that *lat-2* plays an important role in this process. Latrophilin is an adhesion-GPCR that undergoes auto-proteolytic cleavage into the N-terminal and C-terminal fragments, which remain non-covalently associated. It is currently accepted that the dissociation of the extracellular N-terminal fragment exposes the tethered agonist domain in the C-terminal fragment, thereby activating the GPCR signaling. By using CRISPR-Cas9, we generated *lat-2* mutants carrying mutations in the autoproteolytic cleavage site or the tethered agonist domain. Our preliminary data suggest that these mutations do not result in pruning defects, suggesting that *lat-2* function in neurite pruning may not depend on its GPCR activity. In mammals, Latrophilins can also act as ligands of a large type-II transmembrane protein, Teneurin (del Toro et al., 2020; Wang et al., 2024). Consistently, we found that the null mutant of *ten-1(ok641)*, the sole ortholog of Teneurin in *C. elegans*, also exhibits a pruning defect in PDB. We are currently examining the genetic and molecular interactions between *lat-2* and *ten-1* in neurite pruning by examining the pruning defects in the *lat-2; ten-1* double mutants, and in the *lat-2* mutants lacking the C-type lectin domain, which is crucial for Latrophilin-Teneurin interactions in mammals (del Toro et al., 2020). Additionally, we generated the knock-in strains of *mScarlet-I3::lat-2* and *ten-1::mStayGold* to visualize the expression and subcellular localization patterns of endogenous LAT-2 and TEN-1. We are investigating the co-localization patterns in the L2 stage when PDB undergoes neurite pruning. Together, our findings suggest a novel role for Latrophilin and Teneurin in developmental neurite pruning.

810C Comparative analysis of neuronal composition in *P. pacificus* and *C. elegans*

Hyunsoo Yim[1], Yasmin H Ramadan[1], Curtis M Loer[2], Hanh Witte[3], Ralf J Sommer[3,3], Oliver Hobert[11]Columbia University, [2]University of San Diego, [3]Max Planck Institute for Biology Tübingen

The overall structure of nervous system of nematodes is well-conserved across species, yet differences in neuronal number and identity can provide insights into mechanisms of evolutionary adaptations. To address this question, we performed a comparative analysis between *Caenorhabditis elegans* (Cel) and *Pristionchus pacificus* (Ppa), focusing on the neuronal composition of the post-anal tail region. Using pan-neuronal *ceh-48* antibody staining, we found that the number of neurons in this region is reduced from 27 in *C. elegans* to 23 in *P. pacificus*. To further characterize species specific differences in neuronal composition and identity, we used hybridization chain reaction (HCR) (Ramadan and Hobert, 2024) to examine neurotransmitter usage by examining *unc-17* (ACh), *eat-4* (Glu), and *unc-25* (GABA) expression, which define terminal neuronal identity. Additionally, we assessed the expression of key terminal selectors *unc-3*, *unc-86*, and *ceh-14*, transcription factors that regulate neuronal fate, using both HCR and epitope-tagged native loci with immunostaining (Loer et al., 2019). In the post-anal tail region, *unc-3* is expressed in 9 neurons in *C. elegans*, whereas its expression may expands in *P. pacificus*. This highlights species-specific variation in terminal selector expression, suggesting that differences in regulatory mechanisms may underlie neuronal specification divergence between species. Our study highlights both conserved and divergent features of neuronal specification in nematodes and establishes a framework for systematic comparisons of neuronal number, identity, and organization across species.

811C The role of TMEM16F scramblase in neurodegeneration and regeneration

Jialing Yin[1,2], Yang Zhang[2], Chaogu Zheng[1,11]The University of Hong Kong, [2]Shenzhen Bay Laboratory

Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are characterized by progressive neuronal loss, often linked to calcium dysregulation and membrane destabilization. Phosphatidylserine (PS) externalization is a key event in cell clearance, yet its role in neurodegeneration remains unclear. TMEM16F, a calcium-activated phospholipid scramblase, facilitates PS externalization and vesicle formation, but its direct involvement in neurodegeneration is not well understood. Using *C. elegans* as a model, we investigated the role of ANOH-1, the homolog of TMEM16F, in neuronal degeneration and regeneration. We utilized the *mec-4(d)* mutants as a calcium-induced neurodegeneration model, since the dominant gain-of-function mutation in *mec-4(e1611)* animals leads to constitutive calcium influx into the touch receptor neurons and eventually causes them to swell and die from necrosis. By labeling the PS using MFGE8, we observed increased PS exposure on degenerating neurons over time. The loss of *anoh-1* reduced both neuronal degeneration and PS externalization, suggesting a potential role in calcium-mediated membrane destabilization. Importantly, we demonstrated in the HEK293 heterologous system that the *C. elegans* ANOH-1 indeed has the calcium-activated scramblase activity to induce PS exposure. Moreover, we further found that *anoh-1* mutants also exhibited reduced axonal fusion after axotomy, a process that also involves the recognition of externalized PS. Thus, our results suggest that ANOH-1 may play a dual role in both neurodegeneration and neuronal repair. Moving forward, we aim to determine the precise role of TMEM16F in these processes and explore its mechanistic conservation in mammalian models to further investigate the role of TMEM16F in neurodegeneration.

812C Using synthetic cell adhesion molecules to probe the molecular logic of neuronal connections

Jung Hyun Yoo, Maxwell G Heiman Genetics, Harvard Medical School, Boston Children's Hospital

Neurons use cell adhesion molecules (CAMs) to establish connections with the proper partners during development. However, many CAMs are expressed very broadly in the nervous system, raising the question of how they can promote specific contacts. For example, *C. elegans* SAX-7/L1CAM is required for the BAG sensory dendrite to attach to a specific glial partner at the developing nose during dendrite extension, yet SAX-7 is expressed in almost all neurons and glia in the embryo. How do broadly-expressed CAMs like SAX-7 promote specific neuronal connections? To address this question, we are taking a synthetic biology approach using chimeric and synthetic CAMs to define the minimal features needed to promote specific neuronal contacts. We are focusing on three main features: (1) sorting motifs in the intracellular domain (ICD) that control subcellular localization; (2) interaction motifs in the ICD that have been shown to govern the strength of CAM adhesion *in vitro*, for example by coupling adhesion to the actin cytoskeleton; and (3) the extracellular domain that confers binding specificity. Previously, we identified two minimal sorting motifs that control SAX-7 localization in sensory neurons (Lillis et al., Genetics 2022). Here, we examine the role of other interaction motifs in the SAX-7 ICD. Specifically, we tested if defects in BAG dendrite extension in *sax-7* null mutant animals could be rescued by introducing chimeric versions of SAX-7 containing heterologous ICDs from other broadly-expressed CAMs. We found that replacing the SAX-7 ICD with that of its homologs – human L1CAM or *Drosophila* neuroglian – fully rescues BAG dendrite defects. Notably, replacing the ICD with that of the broadly-expressed *C. elegans* neuronal CAMs UNC-40/DCC or NRX-1/Neurexin also resulted in nearly full rescue. By contrast, replacing it with the ICD of the leucine-rich repeat transmembrane protein PAN-1 resulted in no rescue. Our results suggest that yet-undefined functions of the SAX-7 ICD are required to promote specific neuronal connections, and that these functions are shared with the ICDs of divergent CAMs, including UNC-40 and NRX-1, despite the lack of obvious shared sequence motifs.

813C Mitochondrial calcium modulates odor-mediated behavioral plasticity in *C. elegans*

Hee Kyung Lee[1,2], Kyoung-Hye Yoon[1], Kyu-Sang Park[31]Yonsei University Wonju College of Medicine, [2]University of Alabama at Birmingham, [3]Department of Physiology, Yonsei University Wonju College of Medicine

Despite growing understanding of the various roles mitochondria play in neurons, how they contribute to higher brain functions such as learning and memory remains underexplored. Here, using the nematode *Caenorhabditis elegans,* we found that the mitochondrial calcium uniporter (MCU) pore forming unit MCU-1 is required for aversive learning to specific odors sensed by a single sensory neuron, AWCON. MCU-1 expression was required in the sensory neuron at the time of odor conditioning for proper behavioral response to 60 min of prolonged odor exposure. Through genetic and pharmacological manipulation, we show evidence that MCU in AWC is activated in response to prolonged odor conditioning, causing mtROS production, leading to NLP-1 secretion. Finally, we show that the timing of MCU activation and neuropeptide release correspond with the OFF-neuron properties of the AWC neuron, suggesting that mitochondrial calcium entry and neuropeptide secretion coincide with AWC activation upon odor removal. Overall, our results demonstrate that, by regulating mitochondrial calcium influx, mitochondria can modulate the synaptic response to incoming stimuli in the sensory neuron, resulting in learning and modified behavior.

814C Metabolomic analysis of *E. coli* mutants identified potential neuroprotective microbial metabolites

Mengyu Zhang, Chaogu Zheng The University of Hongkong

Neurodegenerative diseases especially Parkinson's disease (PD) have profoundly afflicted humans worldwide, and seeking proper strategies to alleviate the occurrence of PD is critically rewarding. Emerging evidence has revealed that gut microbiome was the pivotal factor influencing PD pathogenesis, in which gut microbiome-derived metabolites as the communicator between brain and gut microbiome might be the key manipulator. We previously established a PD model in *Caenorhabditis elegans* by expressing the human a-synuclein (A53T) in *C. elegans* neurons and screened for *E. coli* genes that promote a-synuclein-induced locomotion defects and neuronal death. This screen led to the identification of 38 pro-neurodegenerative bacteria genes, many of which are involved in metabolism. In the present study, we aimed to identify potential neuroprotective metabolites by analyzing the metabolomes of the *E. coli* mutants that suppressed neurodegeneration in the PD worms. We selected 14 *E. coli* knockout mutants that deleted genes related to metabolism and conducted metabolomic analysis and identified upregulated metabolites in these mutants compared to the wild-type *E. coli*. We then carried out a functional screen of these metabolites, including amino acids, benzenoids, and bile acids, and found five chemicals that showed neuroprotective effects. The five chemicals included two benzenoids (4-Hydroxybenzaldehyde and 3,4-Dihydroxybenzaldehyde), two bile acids, and 5-hydroxy-L-tryptophan. Subsequent analysis showed that these chemicals would reduce a-synuclein aggregation and thus inhibit neurodegeneration in the PD worms. Transcriptomic analysis further identified genetic pathways influenced by the above chemicals, highlighting the potential mechanisms of their neuroprotective functions. Furthermore, some of the chemicals appear to inhibit *E. coli* production of the amyloidogenic curli fiber, which is known to promote a-synuclein aggregation through cross-seeding. Thus, our findings establish novel connections between specific microbial metabolites and PD pathogenesis and reveal their distinct neuroprotective mechanisms.

815C Functional characterization of thermosensory receptor-type Guanylate Cyclases in parasitic nematodes

Yizhe Zhang[1,2], Ben Gregory[1], Astra Bryant[11]Neurobiology & Biophysics, University of Washington, [2]Graduate Program in Neuroscience, University of Washington

Soil-transmitted parasitic nematodes infect over one billion people worldwide and are a major source of neglected diseases. The infective larvae of parasites live in the soil and actively seek out hosts through a poorly understood process involving host-emitted sensory cues, such as heat. Nematode species that have independently evolved into mammalian parasites display a strikingly similar attraction to mammalian body temperature. Here, we test the hypothesis that these independent lineages use similar thermosensory adaptations to enable their shared temperature-driven behaviors.

In *C. elegans*, a cGMP-dependent sensory cascade underlies thermotaxis navigation in physiological temperatures. We previously demonstrated that this thermosensory pathway is conserved in the human parasite *Strongyloides stercoralis* and is essential for temperature-dependent host seeking. In both *C. elegans* and *S. stercoralis*, receptor-type guanylate cyclases (rGCs) generate cGMP in response to changes in temperature. In *S. stercoralis*, three thermosensory rGCs (thermo-rGCs; *Ss-gcy-23.1*, *Ss-gcy-23.2*, and *Ss-gcy-23.3*) display parasite-specific functional adaptations, including expanded responsiveness to mammalian body temperatures. Do similar molecular adaptations occur in other parasite species?

We first investigated the thermo-rGCs of *Strongyloides ratti*, a rat parasite that is closely related to *S. stercoralis*. Surprisingly, compared to all three *S. stercoralis* thermo-rGCs, only one of three rGCs in *S. ratti* (*Sr-gcy-23.1*) conferred thermosensory responses when individually expressed in *C. elegans* non-thermosensory neurons. *Sr-gcy-23.1* exhibited responses that began at human skin temperature and peaked near human body heat, matching the parasite-specific properties of *Ss-gcy-23.1*. Furthermore, we found that co-expression of *Sr-gcy-23.2* and *Sr-gcy-23.1* yielded a lower onset temperature, with responses spanning ambient and mammalian body temperatures, a tuning range that mimics the responses of *Ss-gcy-23.2* alone. These results suggest that the functional properties of thermosensory proteins are partially conserved across parasite species. We are now working to identify and functionally characterize thermosensory proteins of an expanded set of parasitic nematode species, including the human hookworms *Necator americanus* and *Ancylostoma ceylanicum*. Taken together, our results will provide insight into the molecular evolution of sensory systems specialized for parasitism of mammals.

816C A dichotomous role of hydrogen peroxide in modulating the function of the photoreceptor LITE-1

Fengfan Zhang, X.Z.Shawn Xu Life Science Insititute,University of Michigan

LITE-1 is a novel type of photoreceptor protein found in *C. elegans* that is sensitive to short wavelengths of light such as UV and blue light. As illumination of short wavelengths of light generates reactive oxygen species (ROS) such as H2O2, it has been suggested that LITE-1 may also sense H2O2. This notion is reinforced by the fact that LITE-1 is a member of the large invertebrate gustatory receptor (GR) family, whose members are mostly chemoreceptors that sense chemical cues. To address this question, here we interrogated the potential role of H2O2 in regulating LITE-1 function. By recording body-wall muscles ectopically expressing LITE-1 through patch-clamp whole-cell recording, we found that UV light evoked a robust inward cation current, consistent with the notion that LITE-1 is a photoreceptor. By contrast, acute perfusion of H2O2 towards these muscle cells at a concentration up to 500 uM did not evoke any notable current, indicating that H2O2 is unable to directly activate LITE-1. Interestingly, chronic H2O2 treatment revealed a dichotomous effect of H2O2 on LITE-1 function. At low concentrations (e.g. 1 uM), chronic H2O2 treatment potentiated LITE-1 while high concentrations (e.g. 1 mM) of H2O2 inhibited it. We further showed that the potentiation effect of H2O2 required MAPK signaling while the inhibitory effect of H2O2 depended on PRDX-2, revealing a complex role of H2O2 in regulating LITE-1 function. While it remains possible that LITE-1 may be activated by other types of chemical cues, our results help to clarify the role of H2O2 in LITE-1 function and regulation.

817C Functional Interrogation of Neuronal Subtypes via Intersectional Expression of an Optogenetic Actuator Reveals Non-linear Components in a Linear Circuit

Tiannuo Li[1], Sandeep Kumar[2], Hoikiu Poon[1], Andrew M. Leifer[2], Chaogu Zheng[11]The University of Hong Kong, [2]Princeton University

Investigating signal integration in a neural circuit is oftentimes challenging when the circuit contains neuronal subtypes that are transcriptomically similar, due to the lack of tools to express optogenetic actuators with high cellular specificity and to deliver light with high spatiotemporal accuracy. Here, we demonstrate the use of a split GAL4-based genetic “AND” gate to express Chrimson in specific touch receptor neuron (TRN) subtypes in the *C. elegans* touch response circuit. Combining this intersectional strategy for transgene expression with high-throughput optical targeting and behavioral quantification, we optogenetically interrogated the role of each TRN subtype in mediating the mechanosensor-induced escape response and in integrating signals that trigger the opposite motor output. Specifically, we created strains that express Chrimson in only ALM, AVM, and PLM neurons, as well as in combination in all TRNs, in ALM and PLM, and in AVM and PLM neurons. We found that optogenetic activation of the ALM neuron produced a stronger motor output than activating the AVM neuron. More importantly, by comparing the response of the all-TRN, ALM-PLM, and AVM-PLM strains in whole-field stimulation and the targeted head and tail stimulation, we uncovered surprising non-linear components in the touch response circuit. When integrating the anterior and posterior stimuli, the response of the overall circuit linearly combines the competing signals as they were independent of each other in the all-TRN and the ALM-PLM strains. However, in the AVM-PLM strain where the ALM neuron is not activated, we observed non-linear integration of the head and tail stimuli as stronger tail stimulation reduces head sensitivity, while stronger head stimulation increases tail sensitivity. This non-linearity was not observed when ALM is activated, suggesting that ALM and PLM neurons make antagonistic non-linear contributions that cancel out each other, so that they conspire to generate a linear response. Our findings suggest that nonlinearity can exist in seemingly simple neural circuits.

818C Uncover the molecular mechanism of the effect of Proteinopathy on neurodegeneration through oxylipin metabolism

Kin Sing Lee[1,2], Fatemeh YouseFsaber[2], Alan Jamie[1], Morteza Sarparast[31]Pharmacology and Toxicology, Michigan State University, [2]Chemistry, Michigan State University, [3]Michigan State University

Proteinopathy is a neurological disorder driven by the accumulation and aggregation of misfolded proteins. Yet, their molecular mechanism of how proteinopathy is triggered and leads to neurodegeneration remains understudied. Oxylipins are key lipid signaling molecules critical in organismal physiology and pathogenesis of neurodegenerative diseases; but, their role in proteinopathy is largely unknown.

Here, we hypothesize that proteinopathy impacts oxylipin biosynthesis, thus modulating neurodegeneration. To test our hypothesis, we employed *Caenorhabditis elegans* as an animal model. Over the last couple of years, our laboratory has developed multiple tools to investigate the molecular mechanism of the impact of oxylipins on neurodegeneration. Based on our oxylipin analysis in transgenic worms expressing amyloid-beta and tau in neurons, we selected specific oxylipins that are significantly altered and tested them in *C. elegans*. Interestingly, the up-regulated oxylipin induces neurodegenerative phenotype in a dose-response and stereospecific manner. In contrast, the down-regulated oxylipin alleviates the neurodegenerative phenotypes in the transgenic worms that express amyloid-beta or tau in neurons. Lastly, we will present our novel platform to maintain the age-synchronized population of worms for up to 12 days without using 5-fluorodeoxyurdine to sterilize worms, enabling RNAi and compound screening. This platform will help us uncover the molecular mechanism of proteinopathy impacted by oxylipin metabolism.

819C Mapping neuropeptide function with single-neuron resolution

Yiwei Zhou, Pedro Lopes Moreira, Roger PocockDepartment of Anatomy and Developmental Biology, Monash University

Neurons transmit information via two types of signalling molecules: neurotransmitters and neuropeptides. Neuropeptides can act over large distances and have long-lasting effects on animal behaviour and physiology. Neuropeptides are composed of small amino acid chains, which are widely conserved across species. Synthesized neuropeptides are packed into dense-core vesicles (DCVs) and transported to the presynaptic region for release. DCV exocytosis is controlled by the conserved calcium-dependent activator protein for secretion – UNC-31 in *C. elegans*. Released neuropeptides interact with specific G protein-coupled receptors to induce long-lasting effects. *C. elegans* has ~150 neuropeptide genes that generate >300 neuropeptides, however, their neuron-specific functions are incompletely understood.

The fully mapped *C. elegans* neuronal connectome provides an outstanding resource for studying neurotransmitter functions. However, neuropeptides can function independently of synaptic connections and can diffuse over relatively long distances to interact with their receptors. This decentralized mode of signalling makes it challenging to unravel neuropeptide functions.

In this research, we are developing a toolkit to map neuropeptide function with single-neuron resolution. This toolkit uses auxin-inducible degradation of the UNC-31 protein to enable spatiotemporal control of neuropeptide release from every *C. elegans* neuron. To validate this system, we phenotyped animals in which UNC-31 is depleted from the AVK, BAG or DVA neurons. We found that UNC-31 depletion in these neurons caused known defects in animal behaviour (AVK and DVA) and physiology (BAG) known to be caused by neuropeptide loss. Following generation of UNC-31-depletion strains for all *C. elegans* neurons, we will use this toolkit to map neuropeptide functions with single-neuron resolution. This functional map will help the scientific community to better comprehend neuron-specific neuropeptide functions. Additionally, this toolkit has the potential to advance the protein functional studies by enabling the degradation of any protein in any neuron.

820A Box C/D snoRNPs and MDT-15/MED15 regulate innate immunity and mitochondrial surveillance via fatty acids

Lois Armendariz, Elissa Tjahjono, Alicia Chan, Meggie Wang, Natalia V Kirienko Biosciences, Rice University

Mitochondria play key roles in regulating a variety of important biochemical processes that determine cellular health. To maintain homeostasis, multicellular organisms have developed multiple surveillance mechanisms and pathways. Mitochondrial dysfunction is at the core of many diseases, highlighting the importance of clearly understanding mitochondrial surveillance. Our lab focuses on the mitochondrial Ethanol and Stress Response Element (ESRE), which can be activated by reactive oxygen species, mitochondrial damage, and in liquid-based pathogenesis by the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* (LK-*Pa*).

Previously, our lab identified a set of small molecule immune stimulants that increased *Caenorhabditis elegans* survival during exposure to LK-*Pa*. Preliminary data of *C. elegans* treated with LK56, one of these molecules, showed significant enrichment of upregulated lipid metabolism genes, reinforcing a connection between lipid metabolism and innate immunity. Previous studies from other labs have shown that the evolutionarily-conserved mediator subunit MDT-15/MED15 plays a role in initiating an immune response against Gram-negative pathogens, while our data demonstrated that it is also required for LK56-mediated rescue. Interestingly, MDT-15 is also an important regulator for lipid metabolism, as it regulates *fat-6* and *fat-7*, two fatty acid desaturases that convert stearic acid to oleic acid in *C. elegans*' polyunsaturated fatty acid synthesis (PUFA) pathway. However, the mechanism by which lipid metabolism modulates host immunity remains unknown.

We set out to characterize the host lipid metabolism pathways activated by LK-*Pa*. Our lab established that the bacterial siderophore pyoverdine is produced in LK-*Pa* and damages host mitochondria, resulting in a hypoxic crisis and eventual death. We saw that MDT-15 and its downstream effectors, as well as Box C/D snoRNPs (small nucleolar ribonucleoproteins) were required for ESRE activation. Interestingly, supplementation of downstream fatty acids of *C. elegans*' PUFA pathway rescued ESRE activation in their knockdowns. Additionally, we saw that *mdt-15* and *fat-6* RNAi led to decreased worm survival in LK-*Pa*, indicating their role in *C. elegans*' host defense. In addition, our data showed an unexpected requirement for snoRNPs for the activation of FAT-7 following mitochondrial stress. This work will allow us to find ways to modulate ESRE during infection and disease in order to benefit the host.

821A *ppt-1* knockdown impacts lipid droplet regulation and stress response pathways in *C. elegans*

Deborah Ashiedu, Joslyn Mills Biology, Bridgewater State University

Lipid droplets are dynamic organelles essential for lipid storage, metabolism, and cellular homeostasis. Disruptions in lipid droplet regulation have been linked to metabolic disorders and neurodegenerative diseases, yet the molecular mechanisms governing their maintenance remain poorly understood. *C. elegans* serves as an excellent model for studying lipid metabolism due to its genetic tractability and conserved metabolic pathways. In this study, we investigated the role of *ppt-1*, a gene encoding palmitoyl-protein thioesterase 1, in lipid droplet regulation and metabolic homeostasis. Our initial reverse genetic RNA interference (RNAi) screen revealed that there was a decrease in the expression of the lipid droplet-associated protein DHS-3 in nematodes with *ppt-1* knockdown compared to control nematodes of the same strain. We hypothesized that knockdown of *ppt-1* disrupts the depalmitoylation of proteins involved in lipid metabolism, leading to dysregulated lipid homeostasis, increased cellular stress, and shortened lifespan. To test the impact of *ppt-1* knockdown, we conducted a heat stress assay, which unexpectedly showed that *ppt-1* knockdown nematodes exhibited increased survival compared to the control group. This suggests that disruption of protein palmitoylation due to *ppt-1* knockdown may activate stress response pathways that enhance thermal tolerance. We further investigated *ppt-1* function through Western blot analysis to assess chaperone protein expression changes and a thrashing assay to evaluate neuromuscular function to clarify the relationship between *ppt-1*, lipid metabolism, and stress resistance. Gaining insight into *ppt-1* function may help uncover the molecular mechanisms involved in metabolic and neurodegenerative diseases associated with lipid dysregulation.

822A Mitochondrial fusion promoter M1 enhances longevity and mitochondrial function in *C. elegans*

Julia Bailey, Laxmi Rathor, Taylor McElroy, Steven Beck, Sung Min Han University of Florida

Mitochondria play a crucial role in regulating organismal aging and health. Existing studies suggest a correlation between age-related decline in mitochondrial function and aging. Improving mitochondrial activity and homeostasis has been proposed as a potential strategy to mitigate the effects of aging. However, there is currently insufficient knowledge about the effectiveness of pharmacological manipulation of mitochondrial homeostasis in enhancing the lifespan and healthspan of a live animal.

We utilize M1, a small molecule previously found to promote mitochondrial fusion and the expression of mitochondrial fusion-related genes. We employed the model organism *C. elegans* to determine the effect of M1 treatment on longevity and mitochondrial homeostasis *in vivo*. We show M1 treatment extended lifespan in wild-type worms compared to the control. Treatment moderately affected healthspan, with an improvement in motility but no change in overall stress tolerance. Specific markers of mitochondrial homeostasis were altered after M1 treatment, including an increase in membrane potential and a decrease in reactive oxygen species (ROS) levels.

These results provide promising insights into M1 as a potential modulator of animal healthspan and lifespan. Our future goals include investigating the role of mitochondrial fission and fusion proteins in the context of M1 treatment, as well as examining the effects on mitochondrial morphology in the muscle with age.

823A Metformin induced protection against *Streptococcus gordonii* is mediated by SKN-1 via the p38 MAPK and MDT-15

Boopathi Balasubramaniam[1], Ali Al Hatem[2], Alissa Villhauer[1], Ransome Van Der Hoeven[31]Iowa Institute for Oral Health Research, University of Iowa, [2]Iowa Institute for Oral Health Research, University of Texas Health Science Center, [3]Periodontics, University of Iowa

Mitis group streptococci are opportunistic pathogens that reside in the oral cavity and generate hydrogen peroxide (H2O2) as a major contributor to their pathogenesis. Previously, we established that the mitis group mediates killing of the nematode *Caenorhabditis elegans* via H2O2. Further, we have shown that pretreatment of worms with metformin mediates protection from streptococcal-derived H2O2 and is attributed to the activation of the oxidative stress response transcription factor SKN-1/NRF2. Using worms expressing SKN-1 fused to green fluorescent protein, we have shown that the p38 mitogen-activated protein kinase (MAPK) pathway and the transcription mediator complex protein MDT-15 are required for the activation of SKN-1 by metformin. Based on the data, we further evaluated the activation of SKN-1 by p38 MAPK and MDT-15. To determine the activation of the PMK-1 (p38) in response to metformin and the dependence of components of the p38 MAPK pathway NSY-1 and SEK-1, we compared the level of phosphorylation of PMK-1 (p-PMK-1) in wild-type (WT), *nsy-1* and *sek-1* mutant worms pretreated in the presence or absence of metformin by Western blot. We next compared the levels of p-PMK-1 in WT and *mdt-15* worms as described above. Additionally, we performed global label-free proteomics on *mdt-15* mutant worms relative to WT worms in the presence and absence of metformin to identify the differentially regulated proteins. A significant increase in the levels of p-PMK-1 was observed in the presence of metformin which was dependent on *nsy-1* and *sek-1*. However, a significant increase in phosphorylation of PMK-1 was observed in *mdt-15* compared to WT in the presence or absence of metformin. The data suggests *mdt-15* is not epistatic to the p38 pathway but loss of *mdt-15* influences the phosphorylation of PMK-1. Proteomic analysis of *mdt-15* mutant worms relative to WT worms revealed that the expression of many oxidative stress response proteins was influenced by *mdt-15* in the presence of metformin. Collectively, these findings establish that metformin activates SKN-1 through the p38-MAPK pathway and MDT-15 orchestrating a protective response against *S. gordonii*.

824A Investigating the role of *phm-2* and *prpf-4* in *C. elegans* reproductive lifespan

Willie Barron, Lishuang Zhu, Bojun Chen University of North Texas

Scaffold Attachment Factor B (SAFB) proteins are DNA/RNA-binding proteins involved in various cellular processes, including cell growth, stress response, and apoptosis. In *C. elegans*, loss of *phm-2*, which encodes a SAFB homolog, results in a scrawny body shape, extended lifespan, and deceased brood size. To elucidate the molecular mechanisms underlying this phenotype, we conducted a genetic screen and identified two mutants with missense mutations in *prpf-4*, which encodes an ortholog of human PRPF4B, a predicted serine/threonine kinase. Our preliminary data show that *prpf-4* is ubiquitously expressed, with high expression in the gonad. Global knockdown of *prpf-4* via auxin-inducible degradation leads to embryonic lethality, indicating that it is essential for viability. *prpf-4* mutants have normal reproductive lifespan and brood size, and reintroducing wild-type *prpf-4* into the *phm-2;prpf-4* double mutant rescues the reproductive phenotype of *phm-2*, suggesting that *prpf-4* and *phm-2* may function in the same genetic network to regulate reproductive lifespan. Given their roles in gene expression and mRNA processing, we hypothesize that alternative splicing and/or differential expression of genes downstream of *phm-2* and *prpf-4* contribute to the regulation of *C. elegans* reproductive lifespan. Ongoing work aims to identify genes involved in mediating the roles of *phm-2* and *prpf-4* in this process.

825A *C. elegans* RIG-I-like receptor DRH-1 Signals via CARDs to activate anti-viral immunity in intestinal cells

Lakshmi E Batachari, Emily R Troemel Cell and Developmental Biology, University of California San Diego

Upon sensing viral RNA, mammalian RIG-I-like receptors activate downstream signals using caspase activation and recruitment domains (CARDs), which ultimately promote transcriptional immune responses that have been well-studied. In contrast, the downstream signaling mechanisms for invertebrate RIG-I-like receptors are much less clear. *Caenorhabditis elegans* has three RIG-I-like receptor homologs: DRH-1, -2, and -3. While DRH-1 and DRH-3 have roles in anti-viral RNA interference (RNAi), previous work has demonstrated a distinct role for DRH-1 in promoting a transcriptional immune response, known as the Intracellular Pathogen Response (IPR) (Sowa et al., 2020). How DRH-1 signals to activate this transcriptional response remains unclear, as DRH-1 lacks annotated CARDs. In published work, we have demonstrated that DRH-1 has a tandem caspase activation and recruitment domain (2CARD) that promotes IPR gene expression and anti-viral resistance, resembling 2CARD-mediated signaling in human RIG-I (Batachari et al., 2024). Furthermore, we found that DRH-1 is required in the intestine to induce immune gene expression, and we demonstrate subcellular colocalization of DRH-1 puncta with double-stranded RNA inside the cytoplasm of intestinal cells upon viral infection. In unpublished work, we show that 2CARD-mediated activation of the IPR is specific to DRH-1, as the 2CARD of DRH-3, a related RIG-I-like receptor homolog, does not promote IPR activation.

Current efforts are directed at identifying and characterizing signaling factors that function downstream of DRH-1 to activate the IPR. Of note, *C. elegans* lacks known homologs of mammalian RIG-I-like receptor signaling factors, including the mitochondrial protein MAVS. We are developing the tools to perform co-immunoprecipitation mass spectrometry to identify proteins that interact with the 2CARD signaling domain of DRH-1. Findings from this work have the potential to reveal a MAVS-like protein and/or novel anti-viral signaling factors involved in innate immune defense.

826A Thermal stress adaptation in *Caenorhabditis briggsae* and *C. elegans*

Nikita Jhaveri[1,2], Harvir Bhullar[1], Bhagwati P Gupta[11]McMaster University, [2]Johns Hopkins University

The genus *Caenorhabditis* includes several nematode species used in genetic and evolutionary studies, with *C. elegans* serving as the preeminent model organism. *C. briggsae* has emerged as a leading comparative model due to its morphological similarity to *C. elegans* and the availability of a large set of experimental resources to facilitate biological studies. Despite their resemblance, the two species exhibit significant developmental, genetic, genomic, and behavioral differences.

C. briggsae is known to survive and reproduce at higher temperatures more effectively than *C. elegans*. Our lab investigated this thermal tolerance and its genetic basis in detail. We found that *C. briggsae* demonstrates significantly higher heat resistance than *C. elegans*, evident from early developmental stage and extending up to day-one of adulthood. This resilience is shared across tropical and temperate *C. briggsae* isolates, suggesting that elevated thermal tolerance is an intrinsic species-wide trait. To understand the molecular basis of this enhanced thermal resistance, we examined the expression pattern of several heat shock chaperon proteins (hsps). Our analysis revealed that *C. briggsae* mounts a faster and higher heat shock response, with key hsps exhibiting higher induction threshold and rapid recovery compared to *C. elegans*. This suggests that *C. briggsae* has evolved a heightened thermal threshold for molecular stress responses. Interestingly, *C. briggsae* showed greater sensitivity to other stressors, such as oxidative, endoplasmic reticulum, and osmotic stress, indicating a potential trade-off in stress adaptation. Our study provides new insights into the evolution of heat tolerance in nematodes and lays the groundwork for understanding the genetic and molecular mechanisms that shape environmental resilience.

827A Elucidating Context-Dependent Effects of Gut Microbiome Species *Levilactobacillus brevis* on Healthspan and Age-Related Neurodegeneration

Nicole J Braun, Danielle E Mor Neuroscience and Regenerative Medicine, Medical College of Georgia at Augusta University

The gut microbiome has recently attracted considerable attention due to its broad impact on various physiological processes. However, identifying the role of specific host-microbiota interactions and establishing direct causal relationships remains a major challenge. Lactic acid bacteria (L.A.B.) are generally considered uniformly beneficial and are known to undergo diversity changes contributing to dysbiosis in the gut during normal aging and in Parkinson's disease (PD). Yet, how L.A.B. influence host physiology and disease at the singular species level remains largely unknown due to the complexity of the mammalian gut microbiome in tandem with slow aging and low tractability of rodent models. Here we examined the effects of human gut microbiome species *Levilactobacillus brevis* (*L. brevis*) on host health and Parkinson's disease (PD) pathology using *C. elegans*, an ideal model system due to its simple gut anatomy, rapid life cycle, and orthologs for 60-80% of human genes. The unique ability of *C. elegans* to survive on singular bacterial species allows for a highly controlled approach to identify effects of specific species of the human gut microbiome in the contexts of aging and PD. We have found that feeding *L. brevis* to wild-type worms resulted in significant reproductive dysfunction, including severe egg retention and abnormal egg morphologies, due to impaired serotonin signaling in egg-laying neurons. Progeny from *L. brevis*-fed worms showed reduced viability and delayed development, suggesting a decline in reproductive fitness. In parallel, behavioral assays on wild-type worms revealed an age-dependent decline in motor function after *L. brevis* feeding, accompanied by decreased acetylcholine synaptic transmission. Paradoxically, in the context of PD, feeding *L. brevis* to worms expressing human α-synuclein (α-syn) enhanced the survival of dopaminergic neurons, reduced large aggregates and increased the solubility of α-syn, suggesting a potential mitigation of PD pathology. These findings highlight the complex, context-dependent effects of *L. brevis* on host health and demonstrate *C. elegans* as a powerful model for investigating host-microbiota interactions and their implications for human health in the context of aging and age-related disease.

828A Unraveling the pathogenesis of CMT4J using *C. elegans* and advancing drug repurposing efforts.

Constantin Bretonneau[1,2], Claudia Maios[3], Éric Samarut[2,3], Alex Parker[2,31]Neuroscience, CRCHUM, [2]Department of Neuroscience, UdeM, [3]CRCHUM

Charcot-Marie-Tooth disease type 4J (CMT4J) is a rare autosomal recessive neuropathy caused by compound heterozygous mutations in *FIG4*, a phosphoinositide phosphatase crucial for lysosomal function. *FIG4* mutations also contribute to Yunis-Varon Syndrome, ALS, and Polymicrogyria, highlighting the gene's broad pathological significance.

To investigate CMT4J mechanisms and identify therapeutic avenues, we generated *C. elegans figo-1* mutants carrying patient-specific *FIG4* mutations using CRISPR/Cas9. RNA sequencing of these mutants revealed a dysregulation of proteostasis pathways and an increase in oxidative stress response, suggesting impaired cellular homeostasis as a key pathological driver. We are currently validating actionable targets identified through transcriptomic analysis to inform targeted interventions.

Leveraging our *C. elegans* model, we conducted a high-throughput screen of over 4,000 approved drugs. One lead candidate has demonstrated efficacy in rescuing CMT4J-like phenotypes, and we are now advancing preclinical validation in preparation for clinical testing.

829A The role of *dur-1* in dauer stress tolerance

Bailey A. Brown, Capri J. Woss, Madison K. Lester, Jonathan D. Hibshman Department of Biological Sciences, Southern Methodist University

C. elegans is one of few animals that can survive harsh desiccation. The mechanisms that allow *C. elegans* dauer larvae to tolerate the near-complete loss of water from their cells are still unclear, and while some proteins have been reported to be involved in desiccation tolerance, the functions of many of these proteins are still a mystery. DUR-1 is a natively disordered protein that has some homology to LEA proteins—one class of proteins which has been shown to contribute to desiccation tolerance. *dur-1* is upregulated in the dauer stage, and previous research has suggested it may play a role in desiccation tolerance. Yet, little is known about the protein's function during desiccation, as a protectant from other environmental stresses, or more generally in the physiology of the worm. We set out to explore how *dur-1* functions in both normal *C. elegans* physiology and during stress responses, particularly during desiccation. By visualizing DUR-1 with an endogenous fluorescent tag, we confirmed that *dur-1* is significantly upregulated in dauer larvae. DUR-1 has a predicted signal peptide targeting it for secretion. Our initial imaging of DUR-1 in vivo suggests it is likely a secreted protein that may function throughout the body. Working with both a null mutant and a partial deletion of *dur-1*, we have begun characterizing the general health and stress-specific phenotypes associated with this gene. Our preliminary findings suggest that there are *dur-1* allele-specific effects relating to both stress sensitivity and general health, and the *dur-1* partial deletion has the most significant effects on lifespan and reproductive capabilities.

830A Function of the GID Ubiquitin-Ligase Complex in the Control of Metabolism and Organismal Lifespan

Karl E Busch[1], Rakesh Sharma[2], Eliza Karapetian[2], Daniel-Cosmin Marcu[1], Karl Köhnlein[3], Lars-Oliver Klotz[3], Thorsten Pfirrmann[21]Institute for Mind Brain and Behavior, Faculty of Medicine, HMU Health and Medical University, Potsdam, [2]Institute for Molecular Medicine, Faculty of Medicine, HMU Health and Medical University, Potsdam, [3]Institute of Nutritional Sciences, Friedrich Schiller University Jena

Ageing is a process governed by signalling pathways that serve dual roles: they integrate environmental and physiological cues (such as nutrient supply) to regulate cellular energy homeostasis and metabolism, and at the same time they shape organismal lifespan. Increasing evidence suggests a central role of the ubiquitin-proteasome system (UPS) in regulating cellular energy homeostasis, by regulating the turnover, cellular localization, activity, and interactions of metabolic factors. However, it is poorly understood how individual UPS components, through such actions, also contribute to the regulation of ageing.

We discovered an ATP/AMP-independent regulation of AMP kinase (AMPK) activity based on the ubiquitin-ligase function of the vertebrate GID complex. Our analysis shows that the vertebrate GID complex directly regulates AMPK activity. In particular, the GID complex targets p-AMPK for polyubiquitination. Lack of this modification results in constitutive activation of AMPK, independent of the cellular adenylate energy charge. Consequently, loss of GID activity results in enhanced AMPK activity. This causes extensive changes to cellular metabolism, which is visible at the level of the proteome, metabolome and transcriptome.

These observations suggest that the GID complex and AMPK together mechanistically link cellular energy homeostasis with organismal ageing and lifespan. We tested this hypothesis in the model organism *C. elegans* and identified four evolutionarily conserved GID subunits in *C. elegans* (*gid-1, gid-2, gid-7, gid-8*). Strikingly, the RNAi knock-down of each individual subunit resulted in the significant extension of lifespan by more than 30%. In worms carrying *aak-1/aak-2* double deletions, the lifespan extension conferred by RNAi knock-down of *gid-2* disappears. Our data suggest that GID-dependent regulation of AMPK activity is evolutionarily conserved in *C. elegans* and that it underpins the regulation of organismal ageing via AMPK.

831A Ciliogenesis mutants reveal a dauer formation pathway acting in parallel to HSD in the dafachronic acid biosynthesis pathway in *Pristionchus pacificus*

Heather R Carstensen, Anamika Kumari, Talin Ohanes, Sara Honardoost, Ray L Hong Biology, California State University, Northridge

While the dauer larvae as an essential stress-resistant dispersal stage is found in both free-living and parasitic nematodes in Clades 8-12, the functional conservation of genes controlling dauer development across nematode species has not been thoroughly examined. Reverse genetics targeting dauer phenotypes in the genetically tractable nematode *Pristionchus pacificus* revealed that the transcription factor *Ppa-daf-16* and the nuclear hormone receptor *Ppa-daf-12* are functionally conserved in dauer regulation (Daf-d). However, mutation in homologs of Daf-c genes causing constitutive dauer phenotypes in *C. elegans* do not exhibit similar phenotypes in *P. pacificus*, including *daf-19* (ciliogenesis), *daf-11* (guanylyl cyclase), *daf-7* (TGF-β ligand), *daf-3* (Smad), and *daf-4* (TGF-β receptor). Previous work demonstrated that the *P. pacificus* hydroxysteroid dehydrogenase, *Ppa-hsd-2*, is involved in generating dafachronic acids, ligands of DAF-12 that inhibit dauer formation in favorable conditions. Constitutive dauer formation of a *Ppa-hsd-2* null allele can be rescued by exogenous delta-7-dafachronic acid (Δ7-DA). To find additional genes involved in *P. pacificus* dauer regulation, we conducted a forward genetic screen for enhancers of *Ppa-hsd-2*, which form constitutive dauers resistant to exogenous Δ7-DA, and obtained 42 alleles with varying severity. Because some alleles were completely dye-filling defective (dyf), we generated double-mutants of *Ppa-hsd-2* with reference ciliogenesis mutant alleles of *Ppa-daf-19*, *Ppa-che-3*, and *Ppa-osm-1*, and found that they all exhibited constitutive dauer formation that cannot be rescued by Δ7-DA or Δ4-DA. Since dafadine failed to promote the Daf-c phenotype in *P. pacificus,* we also investigated if dafachronic acid production that involves the cytochrome P450 DAF-9 is also functionally conserved in *P. pacificus*. We found that mutants of only one of the three *P. pacificus daf-9* homologs exhibited a strong and persistent Daf-c phenotype, rather than the partial transient dauers observed in *C. elegans daf-9* alleles. These results demonstrate limited ability of genome data to predict conservation of gene function involved in dauer regulation upstream of the steroid hormone biosynthesis pathway in divergent nematode species since the degree of involvement in factors regulating the production of various steroid hormones have also shifted significantly.

832A Sexually transmitted metabolites regulate fat metabolism and fecundity in *C. elegans*

Camila Castellanos-Aldana[1], Bennett W Fox[2], Amaresh Chaturbedi[3], Russell N Burkhardt[2], Diana Fajardo Palomino[4], Jintao Luo[5], Maximilian J Helf[6], Douglas S Portman[7], Frank C Schroeder[2], Siu Sylvia Lee[31]Chemistry and chemical biology, Boyce Thompson Institute and Cornell University, [2]Boyce Thompson Institute and Cornell University, [3]Cornell University, [4]University of Massachusetts Chan Medical School, [5]Xiamen University, [6]5Novartis Natural Products and Biomolecular Chemistry Unit, [7]University of Rochester

Upon transfer during mating, seminal fluid profoundly modifies the physiology and behavior of females across various species. Previous research has identified proteins and RNAs responsible for these effects; however, the potential roles of small molecules in seminal fluid remain largely unexplored. We performed untargeted comparative metabolomics analysis of mated and unmated *C. elegans* to identify metabolites associated with insemination. Our analysis revealed known male-enriched compounds, including a β-methyl fatty acids that are derived from bemeth#1, a β-branched C11 fatty acid and potent NHR-49 agonist that regulates fat metabolism via the fatty acid desaturase *fat-7.* Using bemeth#1-deficient *fcmt-1* mutants, we showed that β-methyl fatty acids can be detected in *fcmt-1* hermaphrodites after mating with WT males, suggesting transfer via the seminal fluid. In support of this hypothesis, *fat-7* expression was increased in animals mated with N2 males but not in animals mated with *fcmt-1* males. In addition, the brood size of animals mated with *fcmt-1* males was significantly reduced relative to that of animals mated with WT males, and this effect was dependent upon *fat-7* expression in the hermaphrodite. Collectively, our study demonstrates that small molecules in seminal fluid influence hermaphrodite metabolism and fecundity, revealing a signaling axis between the sexes that regulates reproductive success.

833A *idh-1* Neomorphic Mutation Impairs Collagen Maturation and Excretory System Development in *C. elegans*

Muthubharathi B Chellammal[1,1], Kaitlin Kaitlin Choi[1], Md Nasful Huda Prince[2,3], Tonmoy Chakraborty[2,3,4], Olga Ponomarova[11]Biochemistry and Molecular Biology, University of New Mexico, [2]Optical Science Engineering, University of New Mexico, [3]Physics and Astronomy, University of New Mexico, [4]Comprehensive Cancer Center, University of New Mexico

Neomorphic mutations in isocitrate dehydrogenase 1 (*IDH1neo*) have been implicated in the development of various cancers. These mutations result in the production of the oncometabolite D-2-hydroxyglutarate (D-2HG), which promotes oncogenesis by disrupting DNA repair, immunometabolic pathways, and the activity of α-ketoglutarate–dependent enzymes. Emerging evidence also points to D-2HG as an inhibitor of collagen prolyl-hydroxylation, a critical step in collagen maturation. To better understand cancer-associated metabolic rewiring, we previously developed a *Caenorhabditis elegans* model carrying the *idh-1neo* mutation. We found that these animals exhibit an unusual sensitivity to vitamin B12, resulting in increased embryonic lethality, and identified the underlying metabolic alterations. Interestingly, *idh-1neo* mutants also develop dilations in the excretory system and show defects in cuticle collagen maturation. In this study, we investigate the mechanisms driving these phenotypes and why they are exacerbated by dietary vitamin B12. Using imaging, genetic interaction analyses, and tissue-specific approaches, we advance our understanding of how *idh-1neo* disrupts normal development and physiological function.

Key words: idh-1neo, D-2-hydroxyglutarate, Collagen maturation, Excretory system, Vitamin B12

834A MXL-3 regulates dietary restriction-induced longevity by transcriptionally controlling SAMS-1 expression

Shih-Jung Chen, Yi-Ang Jung, Tsui-Ting Ching Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

Dietary restriction (DR) has been shown to significantly extend lifespan across a wide range of species, from yeast to mammals, suggesting that the mechanisms underlying DR-induced longevity are evolutionarily conserved. In *C. elegans*, *sams-1* has been identified as a downstream mediator through which DR influences the aging process. *sams-1* encodes a S-adenosyl methionine synthetase, a highly conserved enzyme. Previous studies have reported that DR reduces the mRNA expression of *sams-1*, leading to a decrease in the levels of S-adenosylmethionine (SAM), the metabolic product of SAMS-1, in DR animals. Furthermore, our findings indicate that the overexpression of SAMS-1 can suppress the longevity phenotype observed in *eat-2* mutants, a genetic model of DR in *C. elegans*. The supplementation of exogenous SAM significantly shortened the lifespan of *eat-2* mutants, further supporting the notion that the longevity effects of DR may be mediated through the suppression of SAMS-1 activity. To elucidate how DR modulates *sams-1* transcription, we generated a transgenic worms carrying *sams-1p::GFP*. Utilizing this model for an RNAi-based transcription factor screen, we discovered that the depletion of *mxl-3* led to a significant reduction in GFP expression, suggesting its potential roles in the transcriptional regulation of *sams-1*. MXL-3, a transcription factor in the basic Helix-Loop-Helix (bHLH) family, is homologous to mammalian MAX. Under normal feeding conditions, MXL-3 accumulates in the nucleus of intestinal cells. However, during DR or starvation, it shifts from the nucleus to the cytoplasm, indicating that the activity of MXL-3 is regulated by nutrient availability. Furthermore, our study demonstrated that MXL-3 overexpression ameliorates the reduction of *sams-1* RNA levels in *eat-2* mutants. As knockdown and knockout of *mxl-3* resulted in extended lifespan in wild-type animals, these findings collectively suggest that MXL-3-SAMS-1 axis may play a key role in regulating longevity induced by dietary restriction.

835A Intestinal nuclear hormone receptor NHR-21 mediate stress granule formation induced by starvation

Meng-Chieh Huang[1], Tzu-Shao Liao[1], Tsui-Ting Ching[21]Institute of Biopharmaceutical Sciences, National Yang Ming Chioa Tung University, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

Starvation triggers complex cellular stress responses in organisms. Using single-nucleus RNA sequencing, we investigated molecular mechanisms in *C. elegans* during acute starvation. We discovered that *nhr-21*, a worm homolog of the human HNF4 gene, is specifically upregulated in intestinal cells after two hours of starvation. Moreover, NHR-21 translocates from the cytoplasm to the nucleus during this stress response. RNAi-mediated *nhr-21* knockdown significantly impaired stress granule formation triggered by starvation, suggesting its critical role in starvation response. We further confirmed that *nhr-21* transcriptional upregulation induced by starvation depends on *hlh-30/TFEB*, a master transcription factor of lysosomal biogenesis and autophagy. Moreover, we found that the lipase HOSL-1/HSL is essential for NHR-21 nuclear translocation. HOSL-1/HSL mobilizes fatty acids during starvation, suggesting fatty acids may act as ligands for NHR-21. Indeed, we found that 1 mM oleic acid (OA) induces NHR-21 nuclear translocation even without starvation. In sum, NHR-21 is a novel transcription factor regulating starvation stress in *C. elegans*. Starvation-induced HLH-30/TFEB activation and lipolysis enhance NHR-21 expression and translocation in the intestine, promoting stress response gene expression to maintain fitness.

836A Characterization of the cell-specific transcriptional reprograming in response to OP50 to DA1877 diet shift

Yu-Hsuan Chiu[1], Chung-Yi Liang[1], Tzu-Chiao Lu[1], Tsui-Ting Ching[2], Ao-Lin Hsu[11]Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

Different dietary styles may have a huge impact on many different aspects of physiological functions. Previous studies have demonstrated that both the rate of aging and development are significantly increased when *C. elegans* are fed on *Comamonas* DA1877 compared to those fed on *E. coli* OP50 strain. Our preliminary studies also indicated that transcriptional changes of key metabolism genes, such as *fat-7*, may occur shortly after the OP to DA diet shift. However, how this particular diet shift affects the transcriptome of different cell types in a time-dependent manner remains unclear. Thus, we carried out snRNA-seq to monitor the transcriptional changes in response to the diet shift at several time points. By analyzing the snRNA-seq data, we found that the intestinal expression of *pyk-2* and *alh-8* decreased one hour after the OP to DA diet shift, and then quickly recovered in the second hour. In contrast, intestinal *rpl-7A* expression is continuously increased after the OP to DA diet shift. Further genetic analyses suggest that *pyk-2* and *alh*-8 may act upstream of *rpl-7A* to regulate its expression. Since *rpl-7A* is a gene required for protein translation, we thus speculate that global translation in the intestinal cells may be increased in response to the diet shift. Indeed, results of our puromycin incorporation assay indicate that translation is elevated two hours after the diet shift. Together, our findings suggest that, in response to the OP to DA diet shift, intestinal cells undergo a significant transcriptional reprogramming, which includes a decrease in both *pyk-2* and *alh-8* expression that consequently leads to an increased expression of ribosomal genes and elevated overall protein translation.

837A Oocytes response to heat stress: potential cross talk between P granules and stress-induced RNP granules in the germline

Sumaiya Chowdhury[1], Lisa N Petrella[21]Marquette University, [2]Biological Sciences, Marquette University

The one crucial function of the female germline is to provide viable offsprings. To maintain this function the germline has developed adaptive responses to stressors such as heat stress. One such cellular adaptive response is the assembly of stress-induced ribonucleoprotein (RNP) granules. Stress-induced RNP granules are macromolecular assemblies of RNA-binding proteins, RNAs and stalled translation initiation complexes that assemble only in response to environmental stress. When *C. elegans* is exposed to heat (29-34°C), stress-induced RNP granules start to form in oocytes and the core of the proximal germline. Interestingly, this is the same temperature range when P-granule proteins GLH-1 and PGL-1 decondense fully out of P granules and into the cytoplasm. P granules are germline specific RNP granules and many mutants in P-granule components demonstrate temperature sensitivity fertility defects. Here, we are proposing that decondensation of P-granule proteins and formation of stress-induced RNP granules occurring at the same temperature threshold may be linked through cellular stress response pathways. To test this phenomenon, first, we will block the formation of stress-induced RNP granules either genetically or chemically and then testing how heat stress affects the decondensation of GLH-1 and PGL-1 from P granules. If stress-induced RNP granule formation is important in the decondensation of GLH-1 and PGL-1 from P granules, we predict no decondensation of GLH-1::GFP and PGL-1::GFP in oocytes under heat stress. Then, we will chemically induce stress-induced RNP granules formation at normal temperature and will assess if GLH-1 and PGL-1 decondenses from P granules. We are predicting to observe increased decondensation of GLH-1::GFP and PGL-1::GFP in oocytes. This study will contribute to the knowledge of how structural failure of P granules in *C. elegans* under heat stress may contribute to understand their regulation, organization, and functions, such as their role in fertility at elevated temperatures.

838A UBR-4/KCMF-1, a ubiquitin chain elongating ligase, maintains proteasome robustness

Elyse Christensen[1,2], Nicolas Lehrbach[21]Molecular and Cellular Biology, University of Washington, [2]Basic Sciences, Fred Hutch Cancer Center

Regulated protein degradation via the ubiquitin-proteasome system (UPS) is essential for health and survival. UPS-mediated degradation occurs via multiple rounds of ubiquitination, where the small protein ubiquitin is attached to unneeded/damaged proteins, generating elongated ubiquitin chains that signal for degradation via the proteasome. The regulatory mechanisms that govern poly-ubiquitination to ensure robust protein turnover are not fully understood. Thus, there is a pressing need to understand the mechanisms that govern degradation of ubiquitinated proteins by the UPS and what happens when these mechanisms fail.

We developed genetic tools to investigate novel UPS regulatory pathways that ensure robust protein degradation. Using this toolkit, we performed an unbiased screen in *C. elegans* and identified the highly-conserved E3 ubiquitin ligase UBR-4/p600. Previous studies suggest that UBR-4, in complex with KCMF-1, builds K-48 ubiquitin chains on target substrates, the classic signal for degradation by the UPS. Here, we established *in vivo* evidence that UBR-4/KCMF-1 is required for robust proteostasis during UPS impairment.

Structure function analysis of UBR-4 shows that the UBR-box, the canonical substrate binding domains of UBR-type ub-ligases, is not required for UBR-4's robustness function. However, our study of deletion mutants of both the ubiquitin ligase domain and the ubiquitin-like domain (UBL) shows that they are both required. Consistent with these findings, recent structure preprints indicate that the UBL is necessary to align a ubiquitinated substrate for K-48 chain elongation by UBR-4/KCMF-1.

Physiologically, *ubr-4* null mutant animals exhibit WT growth. However, they show increased sensitivity to the proteasome inhibitor drug bortezomib indicating physiological importance as a robustness factor. Our goal is to connect our findings on the molecular mechanisms required for maintaining robust proteostasis and with physiological function *in vivo.* Such foundational mechanistic insights are a necessary step towards exploring potential therapies to treat proteotoxicity-related illness, such as neurodegenerative disease, and to protect against the effects of natural aging.

839A Modulating material properties of protein condensates to probe exopher cargo inclusion criteria

Edward Chuang[1], Mayur Barai[2], Benjamin Schuster[2], Monica Driscoll[11]Molecular Biology and Biochemistry, Rutgers University, [2]Department of Chemical and Biochemical Engineering, Rutgers University

Propagation of pathological protein species is a hallmark of neurodegenerative disease progression. The mechanism of cell-to-cell transfer and the nature of the pathological protein species that spread in disease are not well understood and are challenging to study in the context of a human brain. We model spreading biology in our study of *C. elegans* exophers, which we hypothesize act as a potential mechanism for pathological proteins to exit the cell.

Exophers are large extracellular vesicles discovered in *C. elegans* neurons and later observed in murine cardiomyocytes, podocytes, and neuronal models. *C. elegans* exophers can contain protein aggregates and damaged organelles. Notably, exophers preferentially recruit overexpressed mCherry and Htt-polyQ128 aggregates while excluding soluble GFP. Selection and recruitment of exopher cargo may be sequence-specific, or it may be a consequence of the aggregation state of the protein. Furthermore, many proteins also form liquid or hydrogel condensates, and it is unknown if exophers preferentially recruit these condensates which have intermediate material properties between soluble and aggregated protein. To address this question, we express PGL-3, a native *C. elegans* germline protein that forms condensates (Putnam NSMB 2019), as a model substrate in our established touch neuron model of exophergenesis. We show that PGL-3 condensates are extruded in exophers, but some PGL-3 can also remain in the neuron. We mutated the intrinsically disordered region of PGL-3 and characterized the altered phase behavior *in vitro* and *in vivo* and measured recruitment to exophers. Our results suggest that condensates with greater viscosity or with viscoelastic (hydrogel-like) properties are extruded in exophers more readily than less viscous liquid condensates. These findings further our understanding of exopher cargo criteria and condensate biology in large vesicle extrusion in neurons of living animals.

840A Elucidating the role of translation inhibition in Caloric Restriction mediated lifespan extension

Catherin DeSousa[1], Bowen Yang[1], Wanyi Zhu[21]Metabolic Biology, University of California, Berkeley, [2]Bioengineering, University of California, Berkeley

Aging is the leading risk factor for numerous chronic conditions, highlighting the need for interventions that can delay the rate of aging and thus the onset of age-related disease. My research focuses on Caloric Restriction (CR), one of the most robust interventions known to extend lifespan and healthspan in a wide array of model organisms. Despite the potency of this intervention to delay the onset of age-related disease, the mechanism has yet to be fully elucidated. Here, I will test the hypothesis that inhibition of translation is, in part, responsible for CR-mediated lifespan extension. Our proteomic studies have shown that the most significant effect of CR on the proteome is the decrease of translational machinery including ribosomes and translation factors, which have previously been shown to extend lifespan in yeast and *C. elegans*. Importantly, we have found that knockdown of translation subunits has a similar lifespan extension as CR, but combining both interventions does not further extend lifespan from either intervention alone. Thus, our data suggests that CR extends lifespan in part through the downregulation of translation. Collectively, these investigations provide fundamental insights into how CR extends lifespan and identify targets for therapeutic interventions to enhance human healthspan.

841A Exploring the impact of Albendazole on *C. elegans* egg stage: A model for understanding soil-transmitted Helminth susceptibility and potential pre-infection control strategies

Giselle Domingo Diaz, Jennifer Domingo, Brian L Ellis Biology, Lipscomb University

Over 1.5 billion of the poorest people are infected with soil-transmitted helminths (STH) such as whipworm, hookworm, and Ascaris which have devastating effects on human growth, nutrition, and cognition, perpetuating the cycle of poverty. The World Health Organization only approves four anthelmintic drugs to treat STH for mass drug administration (MDA), with Albendazole being the drug of choice. Due to these drugs only having two mechanisms of action, resistance to these drugs has been reported over the years. We aim to better understand STH susceptibility to drugs at multiple points in their life stage. Previously, the effects of Albendazole and anthelmintic drugs like Pyrantel, Nitazoxanide, and Ivermectin were tested on L4 and L1 (manuscript submitted) of C. elegans and using a health rating system it showed a health decline, confirming C. elegans as good model organism for parasitic worms (Weaver et al., 2017). In order to investigate how intoxication affected C. elegans at the egg stage, we utilized hatch rates at 5, 10, and 24 hours and LT50 assays with Albendazole in which their health was observed over a seven-day period. It was shown that Albendazole affected hatching rates of C. elegans eggs at the highest dose (100 ug/mL), and using the health rating system from Weaver et al., showed a general decrease in health of worms in all doses. We hypothesize that because the C. elegans are susceptible to drugs at the egg stage, soil could be treated with drugs to control the populations of helminths, even before infection.

842A *pha-4* knockdown reduces mutant HTT aggregation and alters metabolism in *C. elegans*

Francesca Donley, Joslyn Mills Biology, Bridgewater State University

Huntington's disease (HD) is a neurodegenerative disorder associated with mutant Huntingtin protein (mHTT) aggregation in neurons, leading to motor and cognitive decline. In this study, we used *C. elegans* expressing an aggregation-prone mHTT fragment to investigate how the gene *pha-4* regulates mHTT aggregation and overall health.

Knockdown of *pha-4* using RNAi decreases mHTT aggregation. We identified multiple contributing pathways. First, *pha-4* knockdown leads to reduced mHTT gene expression measured by qPCR. Second, pharyngeal pumping assays revealed an increased feeding rate in *pha-4* knockdown nematodes, leading to a greater feeding activity and increased fat stores.

To further investigate the impact of *pha-4* knockdown in the overall health of the nematode, we measured thrashing behavior, fecundity, and heat stress response. Thrashing behavior increased in the second generation of *pha-4* knockdown nematodes, indicating a potential long-term benefit.

The effects of *pha-4* knockdown persisted across generations.The offspring of nematodes with *pha-4* knockdown, who were no longer exposed to RNAi, continued to show lower levels of mHTT aggregation and increased thrashing. Our findings suggest that *pha-4* influences mHTT aggregation and could be a potential therapeutic target for future HD. Surprisingly, we found that *pha-4* knockdown also led to decreased survival under heat stress and reduced reproductivity ability, which may limit its potential as a treatment approach. Further research is needed to understand how *pha-4* interacts with mHTT and whether this approach could be safely applied to human treatment.

843A Investigating the crosstalk between dosage compensation and aging: implications for lifespan and healthspan in *C. elegans*

Joshua Eduful[1], Lily Lesarge[2], Gyorgyi Csankovszki[11]Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, [2]University of Michigan, Ann Arbor

Dosage compensation (DC) is a mechanism that balances X-linked gene expression between sexes in various species, including *Caenorhabditis elegans*, where it is mediated by the Dosage Compensation Complex (DCC). While the role of DC in gene expression is highly studied, its relationship with aging remains largely unexplored. Our study investigates the bidirectional relationship between dosage compensation and aging in *C. elegans*, focusing on its impact on healthspan and lifespan. Our preliminary data reveal that null mutations in *dpy-21*, a key component of the DCC, lead to reduced lifespan, decreased thermotolerance, and impaired locomotion, suggesting that dosage compensation defects accelerate aging. Furthermore, we hypothesize that aging itself affects dosage compensation by altering X chromosome architecture and gene expression. Our preliminary data suggests that X chromosome becomes less compact in aging worms. By investigating the interplay between dosage compensation and aging, our research aims to uncover fundamental mechanisms regulating gene expression, lifespan, and healthspan. These findings may provide broader insights into age-associated chromatin changes and potential interventions to mitigate aging-related decline.

844A Investigating NHR-49 Partner Transcription Factors in Stress and Lifespan Regulation

Stefan Taubert[1], Glafira Ermakova[2], James Wu[2], Kelsie RS Doering[11]University of British Columbia, [2]Medicine, University of British Columbia

Nuclear hormone receptors (NHRs) are a group of *C. elegans* transcription factors (TFs) that regulate many physiological and developmental processes in the organism. NHR-49 regulates lipid metabolism including fatty acid beta oxidation and desaturation. Through this metabolic regulation, NHR-49 is required for the life span of both wild-type and long-lived worm strains. In addition, NHR-49 controls the response to multiple stresses, including to starvation, oxidative stress, hypoxia, and pathogen infection. However, in contrast to other stress response and longevity pathways such as insulin signaling, the *C. elegans* NHR-49 signaling network remains poorly characterized. To identify genes that may act within the NHR-49 signaling pathway, we performed a reverse genetic RNAi screen using the NHR-49-dependent, stress-inducible *fmo-2p::GFP* reporter. This screen identified several TFs which may act within the NHR-49 controlled starvation response pathway. To test whether these genes act with *nhr-49*, I am currently studying several candidate TFs to determine whether their loss or gain of function phenocopies *nhr-49* knockout, i.e., loss causing stress sensitivity and a short life span, and gain causing stress resistance and long lifespan. I am also preforming genetic interaction studies of these genes with *nhr-49* to test whether they act in the same genetic pathway. Thus, my work provides new insight into the makeup and complexity of the NHR-49 stress and longevity network.

845A A high-throughput screening pipeline uncovers novel and repurposed anthelmintics with new mechanisms of action

Hala Fahs[1], Fathima Shaffra[2], Suma Gopinadhan[2], Yasmine Moussa[2], Yamanappa Hunashal[2], Gennaro Battaglia[2], Giselle Cipriani[1], Hin Hark Gan[1], Nabil Rahiman[2], Stephan Kremb[2], Xin Xie[2], Claire Cianca[3], Glenn Butterfoss[2], Gennaro Esposito[2], Rick Maizels[3], Antony Page[3], Kristin Gunsalus[1,2], Fabio Piano[21]New York University, [2]New York University Abu Dhabi, [3]University of Glasgow

Helminths infect an estimated 1.5 billion people worldwide and severely impact livestock and companion animals, making them a major global health challenge. The growing emergence of anthelmintic resistance is alarming, highlighting the urgent need for new treatments with novel mechanisms of action. Using the NYUAD high-throughput robotic screening platform and anthelmintic discovery pipeline, we screened 50,000 compounds with a focus on broad-spectrum activity. This led to the identification of multiple nematicidal small molecules and chemical classes with novel mechanisms of action, highlighting the promise of targeting new pathways for anthelmintic drug discovery. First, we have shown that a set of avocado fatty alcohols/acetates (AFAs) is effective against veterinary parasites both in vitro and in vivo (Fahs et al., Nat Comms 2025; doi:10.1038/s41467-024-54965-w). Genetic and biochemical tests revealed that AFAs inhibit POD-2, an acetyl CoA carboxylase (ACC) that is the rate-limiting enzyme in lipid biosynthesis. Second, another candidate from our screen, ChemAD-16, exhibited potent embryonic and larval lethality in Caenorhabditis elegans and Haemonchus contortus, and it showed both microfilaricidal and macrofilaricidal activity against Brugia pahangi. Third, a family of antifungal compounds displayed nematicidal effects across multiple tested parasites, offering promising potential for repurposing as anthelmintics. All candidate compounds identified in our screens were effective against both anthelmintic-resistant C. elegans strains and field-derived multi-drug-resistant H. contortus (UGA), indicating that they act through novel pathways and targets. These novel compounds offer promising new directions for the development of anthelmintic drugs, either as standalone therapies or in combination. Moreover, targeting multiple pathways simultaneously with drug cocktails may enhance treatment efficacy while potentially helping to mitigate the rise of anthelmintic resistance.

846A Quantifying the influence of phosphagens on the energetics of stress responses

Alyse Wierzbicki[1], Zach Creamer[1], Molly Phillips[1], Cole Dunivan[1], Stephen Kinsey[1], Sarah Fausett[21]University of North Carolina Wilmington, [2]Biology and Marine Biology, University of North Carolina Wilmington

Phosphagen systems are thought to act as both energy buffers and energy shuttles. They consist of a phosphate acceptor such as creatine or arginine, and a phosphagen kinase (PK) that mediates the bidirectional reaction, *phophagen-PO3 + ADP <-> Phosphagen + ATP*. While, much attention has been given to understanding phosphagen systems in tissues that routinely experience bursts of high energy turnover, less has been paid to their potential relevance in other tissues under environmental stress despite mounting evidence that PKs are expressed in diverse tissues and may be major players in orchestrating the stress response. Furthermore, it is becoming clear that phosphagen systems influence not only the immediate energy environment but also downstream signaling events that may support stress response through transcription. To better understand the diversity of roles various PKs might play in the stress response and to more generally explore the energetics of stress responses, my lab is developing a suite of assays that may be combined with stress induced by exposure to media-based agents or hypoxia. Our tools include metabolic flux analysis using the Seahorse Metabolic Flux Analyzer, *in vivo* 31P-NMR, and various endogenous reporters of energy state and mitochondrial function. Combined, these allow us to consider changes in both energy state—including *in vivo* volume averaged concentrations of all high energy phosphates—and energy flux. Our first experiments have confirmed that PK activity is indeed important for maintaining tissue homeostasis under both chronic and acute stress, but that this varies depending on the particular PK isoform. Future experiments will quantify how PKs influence energy state, energy flux, and transcription, and determine whether there are tissue-specific requirements for the various PK paralogs in *C. elegans* under our stress regimes.

847A Transgenerational longevity requires DAF-12 germline-to-soma signaling

Tahreem Fiaz, Scott P Roques, Alexander K Beaudoin, Jaime C Croft, Allison M Sciarratta, Teresa W Lee Biological Sciences, University of Massachusetts Lowell

Generational studies suggest that the experiences of ancestors can affect the health of their descendants, in part by altering how genomes are packaged as chromatin. We have developed a novel *C. elegans* model for transgenerational epigenetic inheritance to examine how heritable chromatin landscapes affect gene regulation and complex traits like lifespan. Mutations in WDR-5 (a component of the MLL/COMPASS H3K4 methyltransferase complex) or JHDM-1 (a homolog of the Epe1 H3K9me2 demethylase) cause the transgenerational accumulation of repressive H3K9me2 in the germline. This increase in repressive chromatin is both necessary and sufficient to extend lifespan by up to 40%, and also mediates the epigenetic inheritance of longevity to wild-type descendants for four generations. Intriguingly, although both *wdr-5* and *jhdm-1* mutant populations eventually attain longevity, they do so with different generational dynamics and striking differences in health.

To examine the mechanism of transgenerational lifespan extension, we test the involvement of DAF-12, a nuclear hormone receptor required for germline-to-somatic signaling. DAF-12 also acts as a key regulator of entry into an alternative larval stage called dauer in response to environmental stress. We find that *daf-12* gene function is necessary for both mutant populations to acquire longevity. Surprisingly, a *daf-12* mutation also suppresses the maintenance of longevity in transgenerational *wdr-5* mutant populations, but not in *jhdm-1* mutants – this result indicates that longevity occurs via different downstream pathways after repressive H3K9me2 accumulation. Finally, mutations in either *wdr-5* or *jhdm-1* partially rescued the dauer-defective phenotype of *daf-12* mutants, suggesting that chromatin landscapes also influence entry into dauer. Taken together, these findings highlight the complex relationship between chromatin state and germline-to-soma signaling during important life history decisions.

848A *fat-3* connects lipid droplet metabolism to fecundity and stress response in *C. elegans*

Mindy Garvin-Leighton, Joslyn MillsBiology, Bridgewater State University

Lipid droplets are dynamic organelles crucial for lipid storage, metabolism, and cellular function. Disruptions in lipid droplet regulation are linked to metabolic disorders and neurodegenerative diseases, but the mechanisms underlying their maintenance remain unclear. *Caenorhabditis elegans* is an ideal model for studying lipid metabolism due to its genetic similarity to mammals and well-mapped genome. In this study, we investigated the role of *fat-3*, a gene encoding Δ6-desaturase essential for long-chain polyunsaturated fatty acid (LC-PUFA) synthesis, in lipid droplet regulation and stress resistance. Using the LIU1 strain expressing DHS-3::GFP as a lipid droplet marker, we hypothesized that *fat-3* knockdown would impair lipid droplet formation, reduce stress resistance, and affect reproductive success due to disruptions in LC-PUFA synthesis.

To test this hypothesis, we conducted stress assays to assess lipid droplet formation, survival rates, stress resistance, and reproductive fitness. Specific stressors included a time course of heat stress at 35°C and an oxidative stress assay using various concentrations of hydrogen peroxide (H_2O_2). Our initial results showed that *fat-3* knockdown led to altered lipid droplet accumulation and reduced stress resistance under heat stress, supporting the role of *fat-3* in maintaining lipid homeostasis under environmental challenges.

This study investigates the impact of *fat-3* knockdown on reproductive fitness and long-term survival in *C. elegans*, providing insights into the molecular mechanisms linking lipid metabolism, stress resistance, and reproductive health. The findings contribute to a deeper understanding of lipid-related diseases, including neurodegenerative disorders and metabolic syndromes.

849A Uncovering key regulators for the mitochondrial ethanol and stress response element surveillance pathway

Sadie Gaskins, Lois Armendariz, Yvette Acevedo, Alicia Chan, Elissa Tjahjono, Armando Moreno, Alexey Revtovich, Natasha Kirienko Rice University

Mitochondria play a key role in maintenance of cellular homeostasis through regulation of several important biochemical processes. Mitochondrial dysfunction contributes to multiple pathologies, including metabolic disorders, cancer, aging, and neurodegenerative disease. Surveillance pathways work to recognize mitochondrial stress by monitoring key mitochondrial functions. When activated, these surveillance pathways initiate transcription of genes that restore cellular function. Previous studies in *Caenorhabditis elegans* showed that the evolutionarily conserved Ethanol and Stress Response Element (ESRE) surveillance network, which acts through an 11-nucleotide motif in the promoter region of target genes, is activated in response to reactive oxygen species (ROS) and mitochondrial damage and is required for defense against *Pseudomonas aeruginosa* in our liquid-based pathogenesis model. Despite ongoing efforts, the regulation of this network remains largely unknown.

We set out to identify potential regulators of the ESRE network by conducting a primary screen on an RNAi library consisting of 1152 transcription factors and kinases using a transgenic worm strain (*3XESRE*::GFP) carrying an array with 3 tandem repeats of the ESRE motif fused to GFP. Rotenone treatment was used to induce *3XESRE*::GFP activation in RNAi-fed worms and fluorescence images were collected at several timepoints. Using this method, we identified 8 repressors and 13 activators involved in ESRE regulation. Bioinformatic analyses of these hits revealed interesting connections between ESRE, ER proteostasis, mitophagy, and worm longevity.

To assess whether our hits were specific to ESRE regulation, we tested their effect on UPRmt and MAPKmt activation under stressed (*spg-7* RNAi) and basal conditions. Interestingly, only 3 of our 21 hits affected activation of these two surveillance pathways, indicating that our pool of hits are generally specific. To assess potential relationships between our hits and UPRER, we will measure UPRER activation by treating RNAi-fed, *hsp-4*::GFP worms with tunicamycin and measuring reporter activity. Further studies will use chromatin immunoprecipitation to differentiate between transcription factors that bind ESRE directly vs. those that affect it via other pathways. Elucidation of a regulatory network for the ESRE pathway will allow us to better understand mitochondrial surveillance and utilize this knowledge to modulate mitochondrial dysfunction.

850A *Caenorhabditis elegans* as a high throughput screening tool for evaluating therapeutic potential of Nano-enabled Antibacterial Combination Therapy (NeACT)

Satwik Majumder[1], Saji George[21]McGill University, [2]Food Science and Agricultural Chemistry, McGill University

Livestock infections involving AMR pathogens is a rising menance for agriculture productivity, animal health and welfare and economy. While combination therapy involving application of two or more drugs of complementary and synergistic actions are being practiced to manage infections, challenges associated with attaining effective concentrations at site of infection, and differential ADME of drugs used reduce the effectiveness. We strategies the use of custom designed nanodelivery platforms to overcome limitations of combination therapy, named as Nano-enabled Antibacterial Combination Therapy (NeACT). C. elegans provide a convenient and high throughput small organism model for screening and optimizing NeACTs targeting intracellular infections. This include evaluating the therapeutic potential of a composite of halloysite nanomaterials with silver and tannic acid and amoxicillin (AMOX) and tazobactam (TAZO)) were encapsulated in custom designed liposome nanosystem containing cyclodextrin core to eliminate intracellular infection with a clinical isolate of multi-drug resistant *Salmonella* Typhimurium (*ST*). Using the cellular and C. elegans model of intestinal infections we were able to demonstrate (a) the reduction in minimal inhibitory concentration of antibiotics in NeACT for resolving intracellular infection involving MDR pathogens, (b) the applicability of using C. elegans in screening nanotherapeutics against intestinal infections.

851A Wild isolate *Bacillus* species may harbor secondary metabolites that antagonize the *Caenorhabditis elegans* Ras-mediated signaling pathway

Emily Washeleski, Paul D Goetsch Biological Sciences, Michigan Technological University

Microbial natural products remain the largest untapped resource for drug discovery, but effectively screening through wild isolate bacteria strains to identify interesting bioactive compounds remains a challenge. To address this challenge, we developed the WormFood Course-based Undergraduate Research Experience (CURE) to screen wild isolate bacteria by feeding them to select *Caenorhabditis elegans* strains. We hypothesized that if a bacterial strain produces a bioactive metabolite-of-interest, then feeding the live bacterial isolate to *C. elegans* will elicit measurable phenotypic effects. To address our hypothesis, we tested for suppression of the *let-60*/Ras oncogenic signaling pathway by feeding ~40 wild isolate strains to *lin-52; lin-8* synthetic mulitivulval (SynMuv) worms. We identified two *Bacillus* strains (*Bacillus safensis* and *Bacillus altitudinis)* out of 11 that suppressed the mulitivulval phenotype. Interestingly, we observed a significantly shorter adult length in worms fed our top *Bacillus* hits compared to OP50 control, suggesting growth inhibition. The two top *Bacillus* hits also displayed a moderate delay in larval development compared to OP50 control, but larval development delay was more pronounced in the other *Bacillus* isolates. Following genome sequencing of all isolated *Bacillus* strains, we were unable to identify evidence that the phenotypic effects were caused by nutritional deficiency. However, since Bacillus species are widely known to produce many secondary metabolites, a search for secondary metabolite biosynthetic gene clusters using antiSMASH revealed that the two *Bacillus* strain hits uniquely contain clusters that may produce metabolites with anti-tumor properties. Therefore, to test whether a metabolite and not nutritional deficiency is likely causing suppression of the multivulval phenotype, we performed intracellular metabolite extraction on the two *Bacillus* isolate hits, using metabolite extraction of OP50 as a control. After overlaying the extractions on OP50 plates, we fed *lin-52; lin-8* SynMuv worms and assessed for suppression of the multivulval phenotype. Importantly, we observed that SynMuv phenotype was weakly suppressed compared to OP50 extract control while adult length was unaffected. We conclude that our WormFood CURE approach to identifying interesting bioactive compounds is effective. We expect that a similar approach could be implemented to screen for novel metabolites with other desired activities dependent on the careful selection of the *C. elegans* genetic or transgenic test strain.

852A AFAs: A new class of natural anthelmintic with a multi-target mechanism

Suma Gopinadhan[1], Hala Fahs[2], Fathima S. Refai[1], Yamanappa Hunashal[1], Gennaro Esposito[1], Kristin C.Gunsalus[3], Fabio Piano[31]NYUAD, [2]NYU, [3]NYU, NYUAD

Nematode parasites pose a significant threat to human health and global food security. The effectiveness of current anthelmintic treatments for the parasites is being compromised by the rise of anthelmintic resistance, creating an urgent need for new drug targets to control these infections. We recently reported a set of avocado fatty alcohols/acetates (AFAs) that exhibit nematocidal activity against all parasitic species tested, including a field-derived multi-drug resistant strain of H. contortus (Fahs et al., Nat Comms 2025; doi:10.1038/s41467-024-54965-w). AFAs also show significant efficacy in mice infected with H. polygyrus. In C. elegans, AFA exposure affects all developmental stages, causing paralysis, impaired mitochondrial respiration, increased reactive oxygen species production and mitochondrial damage. In embryos, AFAs penetrate the eggshell and induce rapid developmental arrest. Genetic and biochemical tests reveal that AFAs inhibit POD-2, encoding an acetyl CoA carboxylase (ACC), the rate-limiting enzyme in lipid biosynthesis. No genetic resistance to AFAs was elicited in C. elegans in extensive EMS screens, suggesting that AFAs may act either through physical damage to biological tissues and/or through multiple genetic mechanisms. Our recent findings indicate that AFAs drive a necrotic form of cell death, leading to neuronal and muscle degeneration. Necrotic death is characterized by elevated intracellular calcium levels and compromised membrane integrity. Genetic disruption studies of necrotic paradigm genes via RNA interference identified gsa-1 (which encodes the Gαs subunit of heterotrimeric G proteins) as a key factor. Disrupting gsa-1 significantly alleviated the AFA-induced death phenotype, suggesting that AFAs may function through the GPCR signaling pathway, in addition to directly inhibiting ACC. We are currently exploring these pathways in greater detail and propose AFAs as promising natural anthelmintics that target multiple mechanisms

853A Extending healthspan in aging nematodes through drug repurposing

Spoorthy Gowda, Ulrike Top Institute of Biochemistry and Biophysics Polish Academy of Sciences

Aging is characterized by a progressive decline in physiological and molecular functions, leading to a poor quality of life for the organism. Healthspan and longevity have been studied in many genetic mutants of *C. elegans*, providing a strong molecular basis for the possibility of organismal rejuvenation. In addition to the analysis of genetic alterations, pharmacological interventions, often through lifelong treatment have shown promising results to prolonging life and healthspan in *C. elegans*. However, genetic and lifelong pharmacological interventions do not seem to be feasible approaches to translate to human biology. This study investigates healthspan modulation in *C. elegans* by repurposing drugs that have been approved for human use and are therefore considered safe. Importantly, drugs were applied to aged worms that had passed the reproductive phase. The treatment of aged animals takes into account changes in molecular pathways that manifest in the hallmarks of aging. We analysed several drugs from different classes including antioxidants, caloric restriction mimetics, metabolites, anticonvulsants and painkillers targeting different molecular processes. We systematically analysed body movement in thrashing assays as a key measure of healthspan improvement. Our results show that specific pharmacological interventions improve neuromuscular coordination and mitigate age-related movement losses, suggesting targeted therapeutic strategies for rejuvenation. Investigation of drug-induced molecular changes will provide important guidance for advancing translational approaches to improve quality of life. This will provide a framework for the use of combinatorial therapies in complex biological systems.

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854A Fitness profiling by RB-TnSeq identifies bacterial genes necessary for *C. elegans* colonization

Olga Perez-Carrascal[1], Lizhen Guo[2], Li-Wen Wang[3], Adam Deutschbauer[3], Michael Shapira[21]Department of Integrative Biology, University of California at Berkeley, [2]University of California at Berkeley, [3]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory

Animals harbor gut bacteria that contribute to their health and fitness. Gut commensalism relies on different bacterial characteristics, including abilities to colonize the gut, to resist host digestion and immunity and to maintain growth. While extensive research has been conducted on bacterial colonization and proliferation in the gut, most studies have focused on pathogenic microorganisms. In contrast, the bacterial factors employed by gut commensals for the same processes remain largely unexplored. We constructed transposon mutant libraries in two worm gut commensals - *Enterobacter hormaechei* CEent1 and *Pantoea cypripedii* V8, and screened them using random barcode transposon sequencing to identify genes required for commensalism in C. elegans. This resulted in identification of 33 genes essential for CEent1 colonization and 49 genes required for V8 colonization. Thirteen genes were required for colonization by both commensals, suggesting evolutionarily conserved bacterial processes that are essential for gut colonization. Additionally, commensal-specific genes indicated differences in metabolic and biosynthetic pathways required by each of the commensals for survival inside the gut. Lastly, candidate genes also include numerous genes with yet unknown links to colonization. These findings underscore the complexity of establishing stable gut colonization by symbiotic bacteria, highlighting the involvement of previously uncharacterized genetic factors in this process. Altogether, our work provides new insights into the bacterial factors that enable host gut colonization and advances our understanding of the mechanisms underlying the establishment and stability of the gut microbiota.

Keywords: Rb-Tnseq, *C. elegans*, colonization, commensal bacteria

855A CEH-60/UNC-62 regulates conditional reversal of aging-like phenotypes by refeeding through UPRER

Ao Guo, Qiang Fan, Meng-Qiu Dong National Institute of Biological Sciences

Aging is manifested by age-associated deterioration of physiological functions that are necessary for fertility and survival. In *Caenorhabditis elegans*, larval-stage starvation induces a diapause state, during which aging-like phenotypes develop gradually at all levels. These aging-like phenotypes can be fully reversed soon after refeeding, underscoring the robust somatic resilience of larvae. However, little is known about the underlying mechanism. To find out, we examined the age at which worms begin to lose this reversal ability. Our findings reveal that when starvation is initiated at the adult stage, refeeding can no longer fully reverse the aging-like phenotypes. Notably, loss of *ceh-60* or inhibition of *unc-62* restores this reversal ability in day 1 adult (AD1) worms. While CEH-60 is known to associate with UNC-62 to activate yolk protein synthesis, our data show that direct inhibition of yolk protein synthesis alone failed to allow AD1 worms to reverse aging-like phenotypes. Since CEH-60 and UNC-62 also repress multiple stress-response pathways, we turned our attention to stress response. Transcriptomic analyses reveal that in *ceh-60* mutant AD1 worms, endoplasmic reticulum unfolded protein response (UPRER) is activated at a higher level upon refeeding compared to wild-type AD1 worms. RNAi knockdown of UPRER genes in *ceh-60* mutants abolished their full recovery from starvation. Furthermore, time-specific overexpression of constitutively active form of XBP-1 (XBP-1s) post-refeeding effectively restores the diminished reversal ability in AD1 worms. Thus, our study posits that adult-onset activation of CEH-60/UNC-62 and the resulting suppression of UPRER are responsible for the loss of somatic resilience in adulthood.

856A Role of the neurotrophic factor MANF in maintaining proteostasis, stress response, and lifespan

Bhagwati P Gupta[1], Shane Taylor[2,2,3,4,5], Jessica Hartman[61]Biology, McMaster University, [2]British Columbia Children's Hospital Research Institute, [3]Centre for Molecular Medicine and Therapeutics, The University of British Columbia, [4]Department of Medical Genetics, The University of British Columbia, [5]Edwin S.H. Leong Centre for Healthy Aging, The University of British Columbia, [6]Department of Biochemistry and Molecular Biology, Medical University of South Carolina

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a conserved protein known for its neuroprotective role and involvement in endoplasmic reticulum (ER) stress regulation. Our lab uses *C. elegans* as a model to study MANF's regulation and function. We have found that *C. elegans* MANF-1 is necessary for the protection of dopaminergic neurons in older adults.

Loss of *manf-1* leads to heightened ER stress sensitivity, reduced lifespan, and increased protein aggregation, underscoring its essential role in proteostasis. In agreement with this essential function, overexpression of MANF-1 extends lifespan, enhances neuronal survival, and reduces toxic protein aggregates in neurodegenerative disease models. Our findings also show that MANF-1 exerts its cytoprotective effects through HLH-30/TFEB, a key transcription factor that regulates autophagy and lysosome biogenesis. Furthermore, we have uncovered the mechanism of MANF-1 function and shown its crucial role in cellular stress responses by regulating autophagy and lysosomal function. We find that MANF-1 localizes to lysosomes and is secreted into the extracellular space, with its transport dependent on endosomal trafficking.

Together, these results establish MANF-1 as a key regulator of proteostasis and cellular homeostasis, with implications for understanding the molecular basis of neurodegenerative diseases and aging. By uncovering a novel link between MANF-1 and lysosomal regulation, our study provides insights into potential therapeutic strategies for proteostasis-related disorders.

857A Post-reproductive gene expression shift in *Caenorhabditis briggsae*: a comparative transcriptomic study

Wouter van den berg, Bhagwati P Gupta Biology, McMaster University

The nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* serve as valuable models for studying genetic pathways governing development, physiology, and aging. Both species are androdioecious hermaphrodites with comparable reproductive spans and brood sizes. However, how they regulate gene expression across reproductive and post-reproductive phases remains largely unexplored.

To investigate this, we performed whole-genome transcriptomic profiling at multiple adult stages. Our analysis revealed a striking pattern in *C. briggsae*: while the majority of differentially expressed (DE) genes were downregulated during reproduction, three-quarters of these genes became upregulated post-reproduction, and similarly a second set of genes shift the opposite way. We have termed this phenomenon the 'post-reproductive shift'. This shift was far less pronounced in *C. elegans*, suggesting species-specific divergence in post-reproductive gene regulation.

Functional analysis of *C. briggsae* DE genes revealed changes in many processes such as the matrisome, muscle development and function, DNA damage repair, stress response, and immune response. Using gene expression assays in mutants and mated animals, we tested whether reproductive status influenced gene expression changes and found in vivo support for the post-reproductive shift.

Overall, our findings uncover a novel species-specific shift in gene expression in *C. briggsae* as animals transition between the reproductive and post-reproductive life stages. The results of this cross-sectional study provide a foundation for further studies investigating the evolution of genetic pathways linking reproduction and aging across nematodes and other metazoans.

858A Combined microplastic and phthalate exposure results in decreased lifespan, reproductive toxicity and stress response in *C. elegans*

Chiara Maldonado, David Mares, Paola Garcia, Maria Gamez, Jennifer Har Biological Sciences, St. Mary's University

Microplastics are plastic particles less than 5 mm in size found in natural and man-made environments. Microplastic are a probable human health threat and have been found in human tissues, including the brain, placenta, blood. Microplastics may contain additives and absorb environmental pollutants that can then be transferred to living organisms after ingestion. After exposure by ingestion, the toxicological pathways and physiological effects of microplastics and combined pollutants are unknown. Here we use *C. elegans* to investigate the effect of Di-butyl phthalate (DBP) and polystyrene microplastic (PS-MP) mixtures on fertility, development, and lifespan. We developed protocols to expose *C. elegans* to DBP PS-MP mixtures when combined in food (OP50 *E. coli*) followed by observation and recording of: brood size (egg laying), abnormalities in eggs (embryo development), lifespan, and stress factors (Daf-16). *C. elegans* were synchronized at larval stage 1 and then they were then exposed to PS-MPs alone, DBP alone and a DBP PS-MS mixture (0.1 M, 3.7 mM, or 100 µM DBP PS-MPs). Worms were continuously exposed to DBP PS-MP mixtures for both lifespan, brood size and stress response assays. To examine the development of eggs and embryos, the same exposures were used, and adult egg-laying worms were bleached to collect internal and previously laid eggs from the plate for imaging by microscopy. While the lower concentrations of the DBP PS-MP mixtures had no significant effect on brood size over PS-MPs alone, a significant reduction in the number of eggs laid per adult worm and embryo hatch fidelity is observed with 0.1 M DBP PS-MPs. DBP PS-MP exposure of the parental worms leads to an increased population of abnormal eggs and non-viable embryos which correlated with DBP concentration level. Chronic exposure to microplastics with and without 0.1 M DBP also reduced *C. elegans* lifespan. Our findings demonstrate that DBP exposed PS-MPs synergistically reduces *C. elegans* fertility, lifespan and leads to a stress response greater than that of either PS-MPs or DBP exposure alone. This work is supported by the NIH NIGMS R16 award R16GM150406.

859A Conserved and novel mechanisms of *Steinernema* colonization by *Xenorhabdus* bacteria

Jennifer Heppert, Heidi Goodrich-Blair The University of Tennessee

Entomopathogenic nematodes in the genus *Steinernema* are colonized by specific beneficial bacteria from the genus *Xenorhabdus.* Together, these species find, kill, and exploit insect prey as a metabolic niche. When nutrients within the insect are depleted, the bacteria recolonize the nematodes in specialized tissues in the anterior intestine in a process called transmission. To understand how animal hosts and their beneficial bacteria recognize each other, we have collaborated to develop genetic and genomic tools in *Steinernema hermaphroditum* and *Xenorhabdus griffiniae.* Using fluorescently labeled *X. griffiniae* bacteria we have observed colonization of the pharyngeal intestinal valve in pre-infective juvenile stage nematodes, and colonization of the receptacle tissue in infective juvenile nematodes. Our findings suggest that multiple *X. griffiniae* cells occupy crypt-like structures within the *S. hermaphroditum* pharyngeal intestinal valve and that the receptacle is ultimately colonized by multiple *X. griffiniae* bacteria. This contrasts with findings from *X. nematophila-S. carpocapsae* where bacteria within the receptacle are derived from one or two progenitor cells, most frequently resulting in a clonal colonizing population within an infective juvenile. *rpoS* is a bacterial sigma factor necessary for *X. nematophila* colonization of *S. carpocapsae*. When we removed the coding region of the *rpoS* homolog in *X. griffiniae,* we observed that the *rpoS* mutant symbiotic bacteria could no longer colonize their *S. hermaphroditum* hosts. These findings suggest that the selective mechanisms occurring at the colonization stage may be both conserved and variable across *Steinernema-Xenorhabdus* pairs.

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861A A label-free high-content imaging assay for thermal stress resistance in *C. elegans*

Thomas J Hodder[1], Chad L Myers[2], Matthew S Gill[11]College of Biological Sciences, University of Minnesota, [2]Computer Science & Engineering, University of Minnesota

Age is a primary risk factor for numerous serious diseases including cancer, diabetes, and heart failure. Thus, therapeutics that slow aging could be instrumental in reducing the burden of these diseases on society. Large scale drug screens for increased lifespan have been carried out in *C. elegans*, but they remain time-consuming and laborious. As an alternative, resistance to acute thermal stress has been shown to be a good predictor of longevity and this relationship has been previously been used to identify drugs that extend lifespan. Screening for thermal stress resistance has several advantages, not least of which is the fact that the assay takes one or two days rather than weeks. However, automated scoring of survival remains problematic. Here we seek to establish a label-free posture-based assay in a 96-well plate format using a deep-learning framework to score survival. Our workflow consists of standard brightfield imaging of worms in a 96 well plate, post-heat shock. Identification and segmentation of worms within each well is achieved using Meta's Segment Anything framework, and classification steps involve fine-tuned Resnet neural networks for image QC and automated determination of live or dead worms. Our proposed assay has the potential to be less cumbersome and more detailed than previously reported worm survival assays, while maintaining the ability to scale to high throughput. So far, we report a consistent way to automatically determine worm survival after a heat shock, the ability to classify worms as alive or dead with accuracy, and the ability to detect interventions that confer heat tolerance. Our automated scoring method is flexible, easy to perform, validated with established techniques, and can potentially lead us to interesting new life-extending interventions and biological mechanisms through unbiased screens.

862A Behavior and genotype of *C. elegans* exposed to simultaneous hypoxia and heat stress

Helen Hong[1], Kwasi M Connor[1], Francesca Dungo[2], Petra Escobar[21]Ecology & Evolutionary Biology, UC Irvine, [2]UC Irvine

Understanding how organisms respond to environmental stressors is crucial for elucidating mechanisms of growth, resilience, and homeostasis. *Caenorhabditis elegans* serves as a well-established animal model for studying stress physiology due to its genetic tractability and conserved response pathways. However, it is emerging as an important environmental model. While heat and hypoxia stress responses have been individually examined, their combined effects under acute exposure remain poorly understood. This study investigates *C. elegans* responses to acute heat and hypoxia stress, both isolated and simultaneously, to assess changes in behavior and genotype. We employ a small-lab friendly hypoxia-heat stress system, designed for precise environmental manipulations, making this technique easily replicable and accessible to the broader research community. Wild-type strain (N2) was exposed to acute mild heat stress (30°C), moderate heat stress (35°C), acute hypoxia (5% O_2), and combined heat-hypoxia stress for a total of 1 hour. Locomotion, feeding behavior, fecundity and egg viability were scored immediately after stress exposure (within 24-48 hours post-stress). Gene expression of stress markers (*hsp-16.2* and *hif-1*) were assessed via RT-qPCR and western blot. We predicted distinct and potentially synergistic effects of combined stress, with differential behavioral and gene expression profiles depending on response to stressors. This study establishes a foundation for hypothesis-driven research on acute stress resilience, providing insights into organismal responses to transient but extreme environmental fluctuations. Furthermore, our methodological approach enables broader accessibility for small-scale laboratories to investigate environmental stress biology.

863A Identification and characterization of *C. elegans* genes that *S. maltophilia* targets to evade host insulin-like DAF-2/16 pathway defenses

Sara Hopkins, Leah Radeke, Michael Herman School of Biological Sciences, University of Nebraska-Lincoln

The bacterivorous nematode *Caenorhabditis elegans* is an excellent model to study host immune responses to bacterial pathogens, such as the emerging human nosocomial pathogen *Stenotrophomonas maltophilia*. Members of the *Stenotrophomonas* genus are components of the *C. elegans* natural habitat and native microbiome (Dirksen et al., 2016; Samuel et al., 2017). Thus, the study of this interaction has both medical and ecological relevance. We have shown that the conserved *C. elegans* p38 mitogen-activated protein kinase (MAPK), transforming growth factor-β (TGF-β), and unfolded protein response (UPR) pathways function to protect the nematode from *S. maltophilia* isolates (White et al., 2016). However, *S. maltophilia* strains JCMS and JV3 are virulent to normally pathogen-resistant *daf-2* mutants, suggesting that pathogenic *S. maltophilia* evades resistance conferred by DAF-2/16 pathway activation. We used transcriptional profiling in *wild-type* and *daf-2* mutants to identify *C. elegans* genes that are significantly differentially expressed in the absence of *daf-2* function upon exposure to *S. maltophilia* as candidates that may be targeted to defeat host defenses. We expect these candidate *S. maltophilia* target genes to be required for *daf-2*-mediated lifespan extension in the absence of the pathogen. To test this hypothesis, we used RNA-mediated interference to knock-down candidate target genes in both *wild-type* and *daf-2* mutant backgrounds and evaluated the effect on lifespan. We found that knock-down of *gale-1* significantly shortens the lifespan of *daf-2* mutants. *gale-1* encodes a UDP-galactose-4-epimerase required for the biosynthesis of UDP-sugars needed for proper protein glycosylation. Reduction-of-function mutations in *gale-1* activate the UPR pathway, possibly by inducing chronic ER stress (Brokate-Llanos et al. 2014). Furthermore, crosstalk between the transcription factors regulating the DAF-2/16 and UPR pathways appears to coordinate ER stress resistance and longevity in DAF-2/16 pathway mutants (Henis-Korenblit et al. 2010). Our work suggests that *gale-1* may link UDP-sugar species biosynthesis, impeded glycosylation, and the UPR pathway in the underlying mechanism enabling *S. maltophilia* to defeat the nematode's DAF-2/16-mediated innate immune defenses.

864A Characterizing the interaction between histone H4 and mtDNA in long-lived animals

Hao-Hsiang Hsu[1], Pei-Yin Huang[2], Tsui-Ting Ching[2], Ao-Lin Hsu[1][1]Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

Heat shock factor-1 (HSF-1) is the master regulator of various cellular stress responses and a key regulator for worm longevity. Overexpression of HSF-1 or downregulation of its negative regulator, heat shock binding protein-1 (HSB-1), has been shown to extend *C. elegans* lifespan. In our previous study, we discovered that histone H4, one of the nucleosome core proteins, translocates into mitochondria and binds to the mitochondria DNA (mtDNA), to be a critical step in the HSF-1/HSB-1 mediated longevity. However, the specific binding region of H4 on mtDNA and the mechanism by which H4 translocates into mitochondria remain unexplored. In this study, we isolated mitochondria and performed mitochondrial DNA immunoprecipitation sequencing (mtDNA IP-Seq.) to identify the specific region occupied by H4 in both wild-type and the long-lived *hsb-1(-)* mutants. The binding patterns of H4 on mtDNA suggest that higher-order structures may exist. We also found that the level of H4 occupancy is significantly increased while maintaining a similar binding pattern in *hsb-1(-)* mutants and heat-shocked human liver cells. Moreover, we found that acetylated H4 is enriched in the mitochondrial fraction.

865A Non-cell-autonomous induction of the endoplasmic reticulum unfolded protein response by COL-75 missense variants

Hung-Jen Shih[1], Jeeyeon Cha[1], James Matthew Ragle[2], Stephane Flibotte[3], Jordan Ward[2], Patrick J Hu[1][1]Medicine, Vanderbilt University Medical Center, [2]University of California Santa Cruz, [3]University of British Columbia

One third of proteins are cotranslationally translocated into the endoplasmic reticulum (ER) during biogenesis. To ensure proper folding of these proteins, a conserved homeostatic program known as the ER unfolded protein response (UPR) has evolved to tune ER chaperone and protein degradative capacity according to unfolded protein load. Disruption of ER homeostasis can lead to ER luminal protein misfolding and aggregation, a condition known as "ER stress" which is associated with common human illnesses associated with aging such as Alzheimer's disease, Parkinson's disease, and diabetes. Thus, understanding the molecular mechanisms that govern ER UPR activation will illuminate the pathogenesis of these aging-related diseases. Whether or how physiologic ER stress in specific cells is communicated organismally is poorly understood.

While studying the conserved ER translocon component TRAP-1, we discovered that a *trap-1* null mutant exhibits constitutive expression of the *hsp-4::GFP* ER UPR reporter. To investigate the role of TRAP-1 in ER homeostasis, we conducted a mutagenesis screen for modifiers of the *trap-1* mutant phenotype (*mtro* screen). The *mtro* screen revealed several known ER UPR components as well as a strain harboring a missense mutation in the *col-75* collagen gene (N162K) that causes constitutive *hsp-4::GFP* expression. A *col-75* nonsense allele does not induce *hsp-4::GFP* expression, indicating that *hsp-4::GFP* induction is not due to loss of *col-75* activity. Induction of *hsp-4::GFP* expression by *col-75(N162K)* requires *xbp-1,* demonstrating that *col-75(N162K)* activates the IRE-1/XBP-1 arm of the canonical ER UPR. Intriguingly, while *col-75* missense alleles induce *hsp-4::GFP* expression specifically in the intestine and spermatheca, an endogenously tagged COL-75 fusion protein is expressed in excretory duct and pore cells and amphid and phasmid socket glia, but not in the intestine or spermatheca. Thus, *col-75* missense alleles activate the ER UPR non-cell-autonomously in the absence of cell-autonomous ER UPR activation. We hypothesize that *col-75* missense variants induce the production of a secreted signal that activates the canonical ER UPR in other tissues.

866A The microsomal triglyceride transfer protein ortholog DSC-4 promotes intestinal endoplasmic reticulum homeostasis by facilitating vitellogenin secretion

Hung-Jen Shih[1], Jeeyeon Cha[1], Stephane Flibotte[2], Patrick Hu[31]Medicine, Vanderbilt University Medical Center, [2]University of British Columbia, [3]Vanderbilt University Medical Center

Loss of proteostasis is a hallmark of aging. Organisms have evolved strategies to maintain proteostasis in response to external cues and internal physiologic state. The endoplasmic reticulum unfolded protein response (ER UPR) is a conserved quality control program that restores homeostasis in response to perturbations that cause intraluminal protein misfolding or lipid bilayer stress (conditions broadly referred to as "ER stress"). ER UPR dysregulation contributes to aging-related phenotypes; its chronic activation is associated with common aging-related diseases in humans; and its enforced activation can extend lifespan. Thus, understanding the contexts, mechanistic basis, and ramifications of ER UPR activation will facilitate the development of new strategies to increase human healthspan by modulating ER UPR activity.

While studying the conserved ER translocon component TRAP-1, we discovered that a *trap-1* null mutant exhibits constitutive expression of the *hsp-4::GFP* ER UPR reporter. To investigate the role of TRAP-1 in ER homeostasis, we conducted a mutagenesis screen for modifiers of the *trap-1* mutant phenotype (*mtro* screen). The *mtro* screen revealed several known ER UPR components as well as a strain harboring a causal splice junction mutation in *dsc-4,* which encodes the *C. elegans* ortholog of microsomal triglyceride transfer protein (MTTP). MTTP is a large lipid transfer protein superfamily member that is required for very low density lipoprotein (VLDL) assembly in and secretion from liver. VLDL has striking similarities to *C. elegans* yolk particles in lipid composition; moreover, APOB, the major apolipoprotein in human VLDL, is structurally similar to the *C. elegans* vitellogenin and major yolk component VIT-2. *dsc-4* mutants exhibit phenotypes consistent with a reduction in yolk assembly and secretion; mutant adults exhibit intestinal retention of a VIT-2::GFP fusion protein, and mutant larvae exhibit developmental delay. We hypothesize that *C. elegans* DSC-4 and human MTTP catalyze lipoprotein complex assembly through a conserved mechanism. The biogenesis of VLDL, yolk, and other similar lipoproteins may play a previously unappreciated role in maintaining ER homeostasis. Understanding how DSC-4 contributes to ER homeostasis promises to illuminate the pathogenesis of lipoprotein-associated human diseases such as fatty liver, Alzheimer's disease, and atherosclerosis.

867A *cnnm-5* regulates multiple pathways of proteostasis in a Huntington's Disease model of *C. elegans*

Matthew Hull, Joslyn Mills Biology, Bridgewater State University

Huntington's disease (HD) is an age-related neurodegenerative disease associated with the aggregation of mutant Huntingtin protein (mHTT). It is theorized that prevention or clearance of these aggregates would protect neurons from degeneration. The maintenance of proteostasis is accomplished by clearing damaged or aggregated proteins which can be carried out by two main pathways: autophagy and the ubiquitin proteasome system (UPS). Macroautophagy, further referred to as autophagy, clears proteins and other macromolecules in bulk by degrading and recycling in an autophagolysosome-dependent manner, while the UPS targets individual proteins tagged with ubiquitin to be degraded by the proteasome. A decrease in functional proteostasis could be driving the Huntingtin proteins aggregations to accumulate, progressing the disease. Using a *C. elegans* model of HD in a small reverse genetic screen of 100 genes on Chromosome 3, *cnnm-5* was identified as a genetic modifier of mHTT accumulation. Since loss of *cnnm-5* by RNAi during development protects against mHTT accumulation, *cnnm-5* may be a negative regulator of protein aggregation clearance. Therefore, we hypothesized that if *cnnm-5* negatively regulates proteostatic machinery, then knockdown of cnnm-5 will increase autophagy and the UPS, which leads to the observed decrease in mutant Huntingtin protein accumulation. Here we report that *cnnm-5* knockdown decreases mHTT protein aggregation, upregulates both major pathways of proteostasis, increases the lifespan of the HD model of *C. elegans*, and protects against paralysis in an Alzheimer's disease model strain.

868A 6-PPD induces mitochondrial dysfunction and reduces healthspan and lifespan through the SKN-1/Nrf2 pathways in *Caenorhabditis elegans*

Moonjung Hyun[1], Laxmi Rathor[2], Taylor McElroy[2], Steven Beck[2], Stephanie Wohlgemuth[2], Rui Xiao[2], Mia Burmudez[2], Erika Hathy[2], Myon Hee Lee[3], Sung Min Han[21]Korea Institute of Toxicology, [2]University of Florida, [3]East Carolina University

The synthetic antioxidant N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD) is commonly used in rubber products, but its toxicity, especially in non-aquatic animals, is poorly understood. We investigated 6-PPD toxicity in mouse embryonic fibroblasts (MEFs) and *Caenorhabditis elegans* (*C. elegans*). In MEFs, 6-PPD induced a dose-dependent increase in cell death, enhanced apoptosis, and impaired mitochondrial function, accompanied by elevated reactive oxygen species (ROS). In *C. elegans*, exposure to 6-PPD resulted in higher mortality, delayed development, reduced reproductive capacity, and a shortened lifespan. Additionally, mitochondrial dysfunction, increased ROS production, decreased mobility, and impaired stress tolerance were observed, highlighting the widespread impact of 6-PPD on cellular and organismal health. Mechanistically, 6-PPD activated SKN-1/Nrf2 signaling, a conserved pathway involved in oxidative stress response. SKN-1/Nrf2 activation was necessary for lifespan reduction and early-stage mortality, but its depletion did not prevent mitochondrial dysfunction, suggesting that mitochondrial dysfunction acts upstream or in parallel to SKN-1/Nrf2 in mediating 6-PPD toxicity. These findings highlight the 6- broad toxic effects of 6-PPD on development, reproduction, healthspan, and lifespan, with mitochondrial dysfunction and SKN-1/Nrf2 signaling playing central roles.

869A Loss of the distal germline GLP-1 activity gradient in aging *C. elegans*

Rustelle J Janse van Vuuren, Patrick Narbonne Medical Biology, UQTR

The local tissue microenvironment around stem cells that acts to maintain their stemness is called the niche. In *C. elegans*, the distal tip cell (DTC) acts as the germline stem cell (GSC) niche by expressing a Notch ligand, LAG-2, that activates the GLP-1 receptor present on adjacent GSCs. As a result, a distal-to-proximal decreasing gradient of Notch activity is established across the progenitor zone (PZ). This single-celled niche provides a simple model for studying stem cells within their microenvironment.

Using single-molecule fluorescence *in situ* hybridization (smFISH) of *sygl-1*, a direct GLP-1 transcriptional target, it was established that Notch transcriptional activity significantly decreased relatively early-on in adulthood (Urman MA *et al*. 2024). Whether this decreased *sygl-1* transcription occurred because of the global transcriptional changes that occur during aging, because of wear and tear of the transcriptional machinery and/or from a specific reduction in Notch receptor activation, remained unclear (Matsuzaki T*et al*. 2024).

Here we used the genetically encoded SALSA (sensor able to detect lateral signaling activity) biosensor (Shaffer and Greenwald, 2022) to examine the pattern of GLP-1/Notch activity across the aging distal gonad *in vivo*. We used the Imaris software to model 3.6 mm diameter spheres over individual GSC nucleus. Sphere fluorescence intensity sums were measured to generate RFP/GFP ratios for each nucleus. To generate a distal to proximal GLP-1 gradient, PZ nuclei were grouped into seven 2-nuclei wide zones from the distal tip.

We looked at PZ GLP-1/Notch activity until the completion of about 67% of the average adult lifespan. Results show that the spatial GLP-1 activation gradient from the DTC, clearly present in young adults, is progressively lost during aging. Thus, in addition to a decline in transcriptional activation, Notch activity also equalizes across the PZ as animals age.

These results may have implications towards several degenerative diseases characterized by perturbations in Notch-regulated SCs, including limbal stem cell deficiency. Abnormal Notch signaling is also evident in various types of cancer and different expression profiles per cancer subtype may result in either tumour suppression or promotion. Apart from tumour progression elevated expression of NOTCH1 has been shown to promote stemness in colorectal cancer stem cells (CSC). Understanding this process is paramount to developing more effective therapies.

870A NIA *Caenorhabditis* Intervention Testing Program: Identification of Robust and Reproducible Pharmacological Interventions That Promote Longevity Across Experimentally Accessible, Genetically Diverse Populations

Monica Driscoll[1], Christine A Sedore[2], Brian Onken[1], Anna L Coleman-Hulbert[2], Erik Johnson[2], Patrick C Phillips[2], Gordon Lithgow[31]Rutgers, The State University of New Jersey, [2]University of Oregon, [3]Buck Institute for Research on Aging

A core facet of the National Institute on Aging's mission is to identify pharmacological interventions that can promote human healthy aging and long life. As part of the comprehensive effort toward that goal, the NIA Division of Biology of Aging established the *Caenorhabditis* Intervention Testing Program (CITP) in 2013. The *C. elegans* model (with an ~21 day lifespan) has led the field in dissection of longevity genetics, and offers features that allow for relatively rapid testing and for the potential elaboration of biological mechanisms engaged by candidate geroprotectants. CITP builds on this foundation by utilizing a genetically diverse set of intervention test strains so that "subjects" represent genetic diversity akin to that that between mouse and humans. Another distinctive aspect of the CITP is a dedicated focus on reproducibility of longevity outcomes as labs at three independent test sites confirm positive outcomes.

The overall goal of the *Caenorhabditis* Intervention Testing Program (CITP) is to identify robust and reproducible pro-longevity interventions affecting genetically diverse cohorts in the *Caenorhabditis* genus. A strong Data Collection Center supports data collection and dissemination. Pharmacological interventions tested by CITP can be nominated by the general public, directed by in-house screens, or supported by published scientific literature. As of December 2024, CITP tested >75 compounds and conducted > 725,000 animal assays over 891 trials. We identified 12 compounds that confer a ≥20% increase in median lifespan to reproducibly and robustly extend lifespan across multiple strains and labs. Five of these interventions have pro-longevity impact reported in the mouse literature (most CITP positive interventions are not tested yet in mouse). As part of the celebration of the 50th Anniversary of the NIA, we review the development history and accomplishments of the CITP program and we comment on translation and the promise of advancing understanding of fundamental aging biology that includes the pharmacological intervention/health interface.

871A Glutamate Ionotropic NMDA Receptor NMR-2 Regulates innate Immunity via Nervous System in *C elegans*

Benson Otarigho[1], Alejandro Aballay[1,21]Genetics, The University of Texas MD Anderson Cancer Center, [2]Microbiology and Molecular Genetics, McGovern Medical School at UTHealth

Glutamate, the primary excitatory neurotransmitter, is implicated in various neurological diseases often linked to inflammation, indicating a potential role in immune modulation. However, the mechanisms by which glutamate signaling regulates host defense remain largely unclear. In this study, we employed reverse genetic screenings to study mutants of genes encoding glutamate receptors, transporters, and enzymes in *Caenorhabditis elegans*. We found that a mutation in *nmr-2*, which encodes an NMDA-type ionotropic glutamate receptor (NMDAR), exhibited enhanced pathogen resistance to *S. aureus* and *P. aeruginosa* without significantly altering intestinal bacterial burden. Furthermore, we found that the enhanced immunity is driven by the upregulation of immune genes through the conserved p38 MAPK/*PMK-1*, insulin/IGF-1/*DAF-16* pathways, and HSF-1/*HLH-30*. We also found that this glutamate-dependent immune regulation acts via some specific sensory neurons (ASE, ASK, AQR, and PQR) as well as AVD interneuron. Our findings uncover a previously unrecognized role of glutamate signaling in suppressing innate immunity.

872B Tetraspanin-Mediated Membrane Resilience and VHL-1 in Heatstroke Survival

Wei Jiang, Dengke Ma Cardiovascular Research Institute, University of California San Francisco [2]Department of Physiology, University of California San Francisco, San Francisco, CA, USA. [3]Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA, USA.*Correspondence: Dengke.Ma@ucsf.edu (D.K.M.)

Our planet is warming, and heatstroke might become a more prominent cause of mortality worldwide, but its pathogenic mechanism is not well understood. We found that early-life thermal stress strongly up-regulates *tsp-1*, a gene encoding the conserved transmembrane tetraspanin in *C. elegans*. TSP-1 forms prominent multimers and stable web-like structures critical for membrane barrier functions in adults and during aging. Up-regulation of TSP-1 is long-lasting even after transient early-life stress. Such regulation requires CBP-1, a histone acetyltransferase that facilitates initial *tsp-1* transcription. Tetraspanin webs form regular membrane structures and mediate resilience-promoting effects of early-life thermal stress1. Moreover, we demonstrate that targeting the VHL-1 (Von Hippel–Lindau) protein can suppress mortality caused by transgenic gain-of-function APOE4 (Apolipoprotein E4), a key genetic risk factor for neurodegeneration in Alzheimer's disease (AD), under heatstroke in *C. elegans*. The protective effects of VHL-1 deletion are recapitulated by stabilized HIF-1 (hypoxia-inducible factor), a transcription factor normally degraded by VHL-1. HIF-1 activates a genetic program that safeguards against mitochondrial dysfunction, oxidative stress, proteostasis imbalance, and endo-lysosomal rupture—critical cellular events linked to mortality2.

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873B Investigating purine degradation inhibition in a *Caenorhabditis elegans* model of ADSSL1 myopathy

Melinda Jin[1], Rishika Patil[2], Wendy Hanna-Rose[21]Biochemistry and Molecular Biology, Penn State University, [2]Penn State University

ADSSL1 myopathy is a rare neuromuscular disorder caused by mutations in the *ADSSL1* gene, which encodes for an enzyme involved in purine biosynthesis, adenylosuccinate synthetase or ADSS. Patients with ADSSL1 myopathy exhibit progressive muscle weakness and atrophy beginning in childhood. As a recently recognized disease in 2016, ADSSL1 myopathy has been poorly characterized and has no standard treatment. Many patients go undiagnosed or misdiagnosed; thus, pre-clinical animal models have significant potential to increase our understanding of the etiology of ADSSL1 myopathy and related diseases.

We have worked on characterizing a loss of function *Caenorhabditis elegans* model for ADSSL1 myopathy in which the *adss-1* gene, the *C. elegans* homolog of the human *ADSSL1* gene, is deleted. We have identified a variety of phenotypes associated with the interference in the function of *adss-1,* such as mobility deficits, sterility, reduced size, and developmental delay. Mobility phenotypes include reduced crawling speed, reduced thrash rate, increased dynamic amplitude, and impaired bending angle regulation. We predict that these phenotypes may result from disrupted purine levels.

In order to prevent further loss of purines in *adss-1* mutant animals, we investigated if inhibition of purine degradation rescues mobility phenotypes. Purines are converted to uric acid by degradative pathways. We targeted the enzyme xanthine dehydrogenase or XDH, which functions twice in purine degradation, converting hypoxanthine to xanthine and xanthine to uric acid. Using RNA interference, we knocked down expression of XDH in *adss-1* mutant animals and assessed mobility.

Preliminary results indicate that reduction of XDH expression by RNA interference results in partial rescue of crawling speed, thrash rate, and dynamic amplitude compared to untreated *adss-1* mutant controls. Additionally, we are investigating the supplementation of *adss-1* mutants with febuxostat, an anti-gout drug and inhibitor of XDH activity. Identifying mechanisms for rescuing mobility phenotypes in our *C. elegans* model may provide us with useful insights into treating patients with ADSSL1 myopathy. Inhibition of purine degradation may be a potential mechanism for addressing ADSSL1 myopathy symptoms.

874B Leveraging *C. elegans* wild isolate variation as a tool to study mitochondrial DNA regulation

Kylie Jozwik, Maulik Patel Vanderbilt University

Unlike the nuclear genome, the mitochondrial genome (mtDNA) is present in tens to thousands of copies per cell and encodes essential protein components of the electron transport chain. At low levels, mutations that arise in mtDNA typically have minimal effects as the remaining wild-type copies are sufficient to sustain cellular energy needs. However, if mutant levels rise beyond a critical threshold, energy needs can no longer be met and mitochondrial disease can arise. While the presence of multiple mtDNA variants—known as heteroplasmy—is common, it remains difficult to predict with certainty the possibility of an individual inheriting a heteroplasmic mutation and its propensity to rise to levels that result in pathogenicity. Since the nuclear genome encodes the protein machinery responsible for mtDNA replication, transcription, and maintenance, previous studies have leveraged human biobank data to identify novel variants responsible for heteroplasmy alterations. However, these studies are inherently correlative, and mechanistic insight is difficult to obtain without testing for direct causality. I aim to overcome these limitations by utilizing the genetically tractable model organism, *C. elegans*, in which wild isolate genetic variation can be systematically interrogated in an unbiased and controlled setting. Using a recently developed genetic tool, I can enforce uniparental inheritance of the nuclear genome while preserving maternally inherited mtDNA. This approach enables precise nuclear swaps across *C. elegans* natural isolates, allowing me to analyze the same mtDNA heteroplasmy in genetically diverse nuclear backgrounds. After identifying strains that differentially regulate heteroplasmy levels, I will chromosomally map the variants responsible and investigate their mechanisms of action. Ultimately, this work seeks to provide a mechanistic understanding of heteroplasmy dynamics which is essential for improving the ability to predict, prevent, and treat mitochondrial diseases.

875B PASH-1 rescues CGG repeat-induced RNA toxicity in a *C. elegans* model of FXTAS

Tsung-Yuan Hsu[1], Noelle L'Etoile[2], Paul Hagerman[3], Bi-Tzen Juang[11]National Yang Ming Chiao Tung University, [2]University of California, San Francisco, [3]University of California, Davis

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an adult-onset neurodegenerative disorder caused by CGG repeat expansions (55-200 repeats) in the 5′ untranslated region (UTR) of the human *FMR1* gene. These expansions result in a movement disorder characterized by tremors, ataxia, cognitive impairment, neuropathy, and autonomic dysfunction, typically manifesting in individuals in their 60s. To investigate the molecular pathogenesis of FXTAS, we utilized *C. elegans* as a model by introducing a single-copy insertion of the human *FMR1* 5′ UTR transgene containing 0, 16, or 99 CGG repeats under a pan-neuronal promoter. Expression of 99 CGG repeats led to age-dependent declines in neuronal function and behavior, mirroring late-onset FXTAS symptoms. RNA toxicity, characterized by elevated levels of repeat-expanded mRNA, has been implicated as a key pathological mechanism leading to RNA sequestration and repeat-associated non-AUG initiated (RAN) translation. Notably, overexpression of PASH-1, the *C. elegans* ortholog of the microRNA (miRNA) processing protein DGCR8, which has a high affinity for CGG-expanded mRNA, effectively mitigated both elevated 99-CGG repeat RNA levels and abnormal FMRpolyG protein accumulation resulting from RAN translation. Furthermore, RNA sequestration-associated miRNA dysregulation was also alleviated. Our findings demonstrate that 99 CGG repeats dysregulate miR-51 expression, leading to the abnormal downregulation of *glo-4* mRNA and subsequent deficits in motor function and neuronal morphology. Importantly, PASH-1 overexpression reversed miR-51 upregulation, restored *glo-4* levels, and rescued motor neuron morphology and crawling ability.

Overall, this study underscores the utility of *C. elegans* as a model for FXTAS research and highlights the critical role of PASH-1 in mitigating RNA toxicity. By reducing elevated CGG-repeat RNA levels and limiting the accumulation of toxic FMRpolyG proteins, PASH-1 overexpression provides a potential strategy to counteract the molecular hallmarks of FXTAS. These findings offer valuable insights into therapeutic approaches targeting RNA toxicity as a means to alleviate neurodegeneration associated with FXTAS.

876B Anti-obesity effects of black rice bran fermented by shiitake in *C. elegans*

Eunyoung Jun, Sangyeong Kim, Seonyu Lim, Chanmi Jang, Kyung Won Kim Life Science, Hallym University

Obesity remains a global health challenge, leading to increased interest in identifying natural products with anti-obesity properties. In this study, we utilized *Caenorhabditis elegans (C. elegans)* as a model organism to assess the anti-obesity and physiological effects of black rice bran fermented by *Shiitake Mycelium* (BRB-F-S). When administered alongside *Escherichia coli* on a high-fat diet starting from the fourth larval stage, BRB-F-S significantly reduced lipid accumulation when observed in 3-day-old adults, as evidenced by Oil Red O staining. Additionally, BRB-F-S improved motility when observed in 5-day-old adults. These findings suggest that BRB-F-S has the potential to modulate lipid metabolism and promote a healthy lifespan. Further studies are needed to identify the active components of BRB-F-S and to elucidate the underlying molecular mechanisms.

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877B H4K8ac interacting ribosomal protein RPL-10 regulates pathogen aversion

Neethu Kamarudheen[1], Alejandro Aballay[1,21]Genetics, The University of Texas MD Anderson Cancer Center, [2]Microbiology and Molecular Genetics, McGovern Medical School at UTHealth

In response to bacterial exposure and intestinal colonization *C elegans* is capable of eliciting a behavioral escape mechanism known as pathogen aversion. We reported that part of pathogen aversion due to intestinal distention is possible due to histone H4 Lys8 acetylation. To identify the potential interacting partners that may affect H4K8ac in response to *Pseudomonas aeruginosa* colonization, we performed ChIP-MS and identified 25 H4K8 acetylated-interacting candidate proteins that were up-regulated more than 3-fold in infected animals. We then hypothesized that these candidate proteins might be involved in pathogen aversion. We found that *rpl-10*, which encodes one of the H4K8ac interacting proteins, RPL-10, is required for pathogen aversion. Because RPL-10 is a large ribosomal subunit protein and similar core host cellular components play a critical role in pathogen response, we are further investigating the rpl gene class to understand the role of this gene family in the process. The study of *rpl-10* and related genes is expected to provide further insights into the connection between intestinal bloating, ribosomal proteins, and gut-neuron regulated aversion behavior.

878B Tissue-specific regulation of the *C. elegans* hypoxia response

Ilona Kesisova, Jina Kong, Bob Horvitz HHMI, Dept. Biology, MIT

Oxygen (O2) is essential for the survival of all aerobic organisms, including *C. elegans,* and the ability to sense and respond to fluctuations in O2 is crucial for life. Hypoxia – low O2 – elicits a wide range of physiological responses at the cellular, tissue and organismal levels. The evolutionarily conserved transcription factor HIF-1 acts as the master regulator of the hypoxia response by promoting a vast transcriptional program necessary for key cellular and organismal adaptations. While the HIF-1 pathway is an extensively studied general driver of the hypoxia response, how different cells and tissues tailor adaptive responses to meet their physiological demands under hypoxic stress remains largely unknown. Our laboratory recently generated a single-cell transcriptomic atlas of the *C. elegans* hypoxia response which revealed that most HIF-1 targets are tissue-specific (Kong et al., 2024, BioRxiv), indicating the existence of mechanisms that drive the HIF-1 transcriptional response in a cell- and tissue-specific context.

To seek genes that regulate the cell- and tissue-specificity of the hypoxia response, we performed genetic screens using broadly induced or tissue-specific reporters of HIF-1 activation as proxies for the HIF-1 transcriptional output. We hypothesize that there are genes that selectively repress the expression of tissue-specific HIF-1 effectors in non-target tissues and/or genes that selectively activate the expression of tissue-specific HIF-1 effectors in target tissues but not in all tissues. Thus far we have performed screens using two broadly induced and three tissue-specific reporters, and we have isolated nine candidate mutants. Five of these isolates selectively repress the normally broadly induced HIF-1 target *nhr-57* in body-wall and pharyngeal muscles. Four isolates selectively repress the normally muscle-induced *pcca-1* expression in body-wall but not pharyngeal muscles. (While screening for tissue-specific reporters of HIF-1 activation, we identified *pcca-1* as a novel muscle-specific HIF-1 effector.) We are confirming these candidates and seeking their causative mutations. We also are continuing screens with the same and additional reporters. We hope that our work will help answer the fundamental question of how the ubiquitous and evolutionarily conserved HIF-1 transcription factor mounts distinct tissue- and/or cell-specific transcriptional responses and how such responses drive tissue-specific adaptations to low oxygen.

879B Investigating the effects of dietary restriction and genetic variation in lifespan using novel recombinant inbred lines of *C. elegans*

Sehrisha Khan[1], Lisa van Slujis[2], Joost Riksen[2], Jan Kammenga[2], Mark Sterken[2], Marieke Bloemink[1], Jana Stastna[11]Canterbury Christchurch University, [2]Wageningen University

Ageing is a universal phenomenon during which continuous changes ultimately lead to death. One of the conditions that often accompanies the ageing process is neurodegeneration. The progressive loss of structure or function of neurons often lead to cognitive, motor, or sensory impairments and can severely impact the quality of life. Damage due to protein accumulation is one of the suspected causes. Here, we study a protein-accumulation model of *Caenorhabditis elegans* which accumulates human α-synuclein. We investigate the role of nutritional status on protein accumulation and lifespan.

Recently, we constructed a novel recombinant inbred line (RIL) panel derived from a cross between genetically divergent strains *C. elegans* NL5901 and SCH4856, both harbouring the human α-synuclein (αS) gene; αS-RILs. This panel has been investigated for gene expression and has been genotyped by whole-genome sequencing. Here, we measured lifespan and the effect of food restriction via peptone deprivation on ~90 αS-RILs under both normal and dietary restrictive (DR) conditions.

Preliminary data from this research indicate great variability in lifespan across the αS-RILs, with an overall increase in lifespan under dietary restrictive conditions. However, we also find a substantial number of RILs that did not follow the usual DR paradigm of extended lifespan. These results indicate that peptone deprivation mediated dietary restriction affects lifespan in *C. elegans* in a genotype-dependent manner.

880B Taurine as a promoter of healthy aging via dietary restriction signaling in *C. elegans*

Sangyeong Kim, Chanmi Jang, Kyung Won Kim Life Science, Hallym University

While scientific advancements have increased human lifespan, they do not necessarily ensure healthy aging. Taurine, a naturally occurring amino acid, has emerged as a promising candidate for promoting both lifespan and healthspan. Building on the findings of Singh et al. (2023), which demonstrated taurine's longevity-enhancing effects in *C. elegans* and mice, our study investigates its underlying mechanisms. We confirmed taurine's lifespan-extending effects in *C. elegans* using both live and dead *E. coli* as food sources. Additionally, taurine supplementation improved key markers of healthy aging, including intestinal barrier integrity, locomotion, sensory perception, and long-term memory retention. These physiological benefits suggest its role in mitigating multiple age-related declines. To elucidate the molecular basis of these effects, transcriptome analyses revealed that taurine modulates genes associated with dietary restriction (DR) signaling. Using *eat-2* mutants, a genetic model of DR, we found no additive lifespan extension, indicating a shared longevity pathway. Furthermore, taurine reduced lipid accumulation, a hallmark of DR, without altering pharyngeal pumping rates, suggesting metabolic alterations rather than direct caloric restriction as the primary mechanism. These findings establish taurine as a potent regulator of both lifespan and healthspan, highlighting its potential benefits in combating age-related conditions.

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881B Resistance profile of Cry proteins combating plant parasitic nematodes

Youmie Kim[1], Sara Lenzen[2], Kelly Flanagan[1], Julia Daum[2], Jayme Williams[2], Raffi V Aroian[11]UMASS Chan Medical School, [2]BASF

Bacillus thuringiensis (Bt) has been successfully used commercially for more than 60 years for biocontrol of insect pests. Among the hundreds of Cry proteins classified into more than 50 families, some—including Cry5Ba and Cry14Ab—exhibit nematicidal activity and share structural similarity with insecticidal Cry proteins.

Cry5Ba, when expressed in transgenic tomato roots, provided control over infection by the root-knot plant-parasitic nematode (PPN) *Meloidogyne incognita*. The nematode *Caenorhabditis elegans* has served as a model organism to dissect the molecular mechanisms of Cry protein toxicity. Cry5B binds to glycosphingolipid receptors on intestinal epithelial cells, resulting in pore formation and cell lysis. *bre-2*, *bre-3*, *bre-4*, and *bre-5* (for ***B***t protein ***re***sistant) encode glycosyltransferases responsible for constructing a galactose-rich glycolipid receptor essential for Cry5B binding. Therefore, *bre* mutants are specifically highly resistant to Cry5B.

The soybean cyst nematode *Heterodera glycines* is a major agricultural pest causing over $1 billion in annual yield losses in the U.S. Transgenic soybeans expressing Cry14Ab—now approved by both the EPA and FDA—exhibit resistance to *H. glycines*. To better understand Cry14A resistance, we conducted genetic screens in *C. elegans*. Although non-conditional screens did not yield highly resistant mutants, conditional (temperature-sensitive) screens identified two mutants, *bre-6(ye123)* and *bre-7(ye124),* that exhibit resistance to Cry14A family proteins.

The *bre-6* mutant was identified as the nuclear hormone receptor *nhr-31* by RNAi, DNA rescue, and CRISPR analyses and was renamed *nhr-31(ye123)*. *nhr-31(ye123)* animals showed significantly reduced expression of most of the subunits of the *C. elegans* vacuolar ATPase (vATPase) and reduction in vATPase subunits resulted in resistance to Cry14A family proteins, albeit with a high fitness cost.

In evaluation of new Cry proteins targeting plant-parasitic nematodes, we evaluated the resistance profiles of *C. elegans* strains (N2, *bre-4(ye13)*, *bre-6(ye123)*, and *bre-7(ye124)*) against four newly engineered Cry proteins, alongside Cry5B, Cry14Aa, and Cry14Ab. Here, we will report on the resistance profiles of these *C. elegans* mutants to this panel of Cry proteins.

882B Calcium homeostasis regulates Urolithin A-induced mitophagy to promote healthspan and lifespan

Aikaterini Kitopoulou[1], Antonis Roussos[1], Fivos Borbolis[1], Christina Ploumi[1], Haijun He[1], Martina Samiotaki[2], Konstantinos Palikaras[11]Department of Physiology, Medical School, National and Kapodistrian University of Athens, [2]Biomedical Sciences Research Center "Alexander Fleming", Institution for Bioinnovation

Mitochondrial dysfunction and mitophagy impairment are hallmarks of ageing and age-related pathologies. Ageing is also marked by disrupted inter-organellar communication, yet how mitophagy can restore this communication remains poorly understood. Here, we show that urolithin A (UA), a potent mitophagy-inducing molecule, restores inter-organellar crosstalk via calcium signaling, which is critical for mitophagy activation and cellular homeostasis in *C. elegans*. Our transcriptomic and proteomic analyses reveal that UA reorganizes interactions among the endoplasmic reticulum (ER), lysosomes, and mitochondria, with cytosolic calcium dynamics playing a central role. UA induces calcium release from the ER, enhances lysosomal activity, and drives DRP-1-mediated mitochondrial fission, culminating in efficient mitophagy. Calcium chelation abolishes UA-induced mitophagy, resulting in impaired muscle function and reduced lifespan extension, underscoring the critical role of calcium signaling in UA's geroprotective effects. Our findings uncover the molecular mechanism by which UA-induced mitophagy restores inter-organellar communication, supporting energy metabolism and organismal health.

883B The *C. elegans* pre-dauer L2 prematurely restricts germline growth in response to nutrient deprivation, correlated with reduced reproductive success

Fred A Koitz, Kacy Lynn Gordon Biology, University of North Carolina at Chapel Hill

The C. elegans dauer suspends development in crowded or nutritionally inadequate environments unsuitable for reproduction, yet the reproductive system is less well-studied than other dauer organ systems. Extended diapause has been reported to diminish post-dauer (PD) reproductive success, but there are conflicting results and potential mechanisms are unknown. We find that dauer formation-constitutive (daf-c) mutants that form dauers in the absence of natural dauer-inducing cues do not see reduced reproductive success after prolonged dauer, suggesting that time in dauer alone cannot explain PD reduced fertility. Using live imaging of transgenic animals with reproductive system markers, we compared the reproductive system morphology of wild-type dauers on recently-starved plates versus those starved for over a month. While both maintain quiescence independently of GLP-1/Notch and express comparable amounts of LAG-2/Delta in the DTCs, we were surprised to find that the reproductive system morphology of recently-formed dauers differs compared to animals on older plates, with most dauers on older plates having a dramatically reduced germline (length and cell number) and a more compact somatic gonad (reduced size but not cell number). The daf-c insulin-like signaling mutant *daf-2(e1370)* also sees this difference after a month when dauers are formed by starving at the permissive temperature, but constitutive dauers of the same genotype have stable reproductive system morphology over an extended diapause. In line with this observation, *daf-2(e1370)* has a PD brood size reduction comparable to wildtype only when dauer is induced naturally. We hypothesized that the reduction in gonad and germline size observed after natural dauer-forming conditions reflected either germline shrinking over extended diapause or differences in pre-dauer germline growth. After ruling out mechanisms that reduce germ cell number in other developmental contexts, we turned our attention to pre-dauer conditions that might limit germ cell proliferation or longevity. Our results implicate pre-dauer nutrition in the gonad size difference, but find that insulin-like signaling is surprisingly dispensable for this nutritional cue. Together, these data suggest that the post-dauer reduction in fertility seen over time is not alone a result of time in dauer itself, but is instead dictated by the pre-dauer environment, showcasing how early-life adverse events can have long-lasting effects.

884B Transgenerational adaptive behaviour and immune priming in *C. elegans* upon *Salmonella* Typhi infection via dopaminergic and insulin signalling

Balamurugan K Krishnaswamy Biotechnology, Alagappa University, INDIA

Pathogenic infections can induce long-term behavioural and immune changes in hosts. Using the model organism *Caenorhabditis elegans*, we demonstrate that a single parental exposure to the pathogen *Salmonella enterica* Serovar Typhi (*S.* Typhi) induces a specific transgenerational avoidance behaviour persisting for up to three generations. This response was pathogen-specific and absent with non-pathogenic bacteria.

The offspring of the *S.* Typhi infected parents exhibited enhanced survival and egg laying potential upon re-exposure. Molecular analysis revealed upregulation of C-type lectins (*clec-60, clec-67, clec-87*), suggesting immune priming and activation of the insulin/IGF-1 signalling pathway marked by *daf-2* downregulation and *daf-16* upregulation indicating the possible role of the IIS pathway mediated immune protection. Subsequent experiments with chemical induced inhibition of dopamine cascade and *dat-1* mutant based studies suggested that the involvement of dopamine signalling in the transmission of the learnt avoidance trait.

Interestingly, with continuous multigenerational exposure, worms displayed an altered dopaminergic expression in CEP, ADE, and PDE neurons suggesting neuronal modulation during the inherited behavioural changes. Metabolomics analysis of parental and off-springs suggested the transmission/modulation of host specific regulatory metabolites during the transgenerational exposure with *S.* Typhi.

Although, the preliminary data suggested the involvement of dopaminergic signalling as one of the crucial players for transmitting pathogen-associated experiences, the role and contribution of other signalling pathways during this adaptive behaviour along with other transmitting small molecules from one generation to next will offer a clear perspective on how a multicellular organism adapt over time against recurring environmental threats.

885B Learned Aversion of *Chryseobacterium*, a Natural *C. elegans* Pathogen

Yuan-Chen Kuo[1], Yu-Chun Wu[1], Chun-Liang Pan[21]Institute of Molecular Medicine, College of Medicine, National Taiwan University, [2]National Taiwan University

Aversive learning for pathogens, predators or environmental hazards is important for the survival of animals. Our knowledge of the molecular mechanisms underlying aversive learning comes almost exclusively from laboratory studies using a few model systems. Establishment of alternative models is important for a more comprehensive understanding of the molecular processes governing learning and memory. The nematode *Caenorhabditis elegans* develops learned avoidance of bacteria under concomitant tissue stress. We recently discovered that the sphingosine kinase SPHK-1 and its product, sphingosine-1-phosphate (S1P), promotes aversive learning under mitochondrial stress triggered by inhibitors of mitochondrial respiration. Although *Pseudomonas aeruginosa* causes mitochondrial stress in *C. elegans*, *sphk-1* is not required for the aversion of *Pseudomonas*. Interestingly, learned aversion of *Chryseobacterium indologenes*, a little-studied aerobic rod pathogen for *C. elegans*, requires *sphk-1* and S1P. *sphk-1* acts in peripheral tissues, such as the intestine and hypodermis, hinting on a body-to-brain pathway for aversive learning. Using candidate mutant screens, we find the serine *C*-palmitoyltransferases *sptl-1* and sphingosine *N*-acyltransferases *lagr-1*, two enzymes required for the production of sphingosine, are required for learned *Chryseobacterium* avoidance. Our genetic analysis indicates that GABA and neuropeptides play important roles in *Chryseobacterium* avoidance, and several monoamine neuromodulators, including serotonin, dopamine and octopamine, play additional roles. We are currently in the process of mapping additional genes for the sphingolipid biosynthesis, as well as neurons and circuits for GABA and neuropeptide signaling underlying *Chryseobacterium*-associated learning. (Supported by National Science and Technology Council NSTC 112-2320-B-002-018-MY3)

886B Temporal and spatial regulation of the autophagy-regulating transcription factor HLH-30/TFEB in hormesis and aging

Cheng-Ju Kuo, Tatiana Moreno, Diego Hernandez, Caroline Kumsta Sanford Burnham Prebys Medical Discovery Institute

Autophagy is a crucial cellular process that maintains proteostasis by degrading and recycling unwanted components, including protein aggregates and damaged organelles. However, autophagic activity declines with age, leading to age-related diseases. Enhancing autophagy has shown promise in delaying aging and mitigating related diseases. We discovered that exposing *Caenorhabditis elegans* to mild, non-lethal heat shock (HS) induces autophagy, enhances proteostasis, and extends lifespan through hormesis, a phenomenon where low-dose stress elicits beneficial effects. The autophagy master transcription factor HLH-30/TFEB plays a pivotal role in this process, translocating to the nucleus upon HS and driving autophagy induction. Both autophagy and *hlh-30*/*Tfeb* are required for hormetic benefits. This raises critical questions: How does the transient nuclear localization of HLH-30/TFEB translate into sustained benefits? Does HS trigger a temporary autophagy response that is sufficient for long-term health advantages, or does it induce lasting changes in autophagy regulation to support hormesis? Since we observed tissue-specific differences in HLH-30/TFEB nuclear translocation kinetics following HS, we hypothesize that tissues have distinct autophagy gene induction patterns, which may influence hormetic outcomes. To address whether autophagy plays tissue-specific roles in hormesis and how HLH-30/TFEB contributes to hormetic benefits in specific tissues, I employed the auxin-inducible degradation (AID) system to dissect the temporal and spatial requirements of HLH-30/TFEB for hormesis. Our results show that whole-life and ubiquitous loss of HLH-30/TFEB abolishes HS-induced benefits, mimicking the effects seen in *hlh-30* mutants. Preliminary data suggest that HLH-30/TFEB in neurons and intestines, but not in muscles, is required for hormesis. The AID system will also help to determine the temporal requirement of HLH-30/TFEB during hormesis. Furthermore, to gain deeper insights into HLH-30/TFEB regulation, I am conducting single-cell RNA sequencing (scRNA-seq). I developed a cell dissociation protocol and used flow cytometry to isolate somatic cells for scRNA-seq. This study offers the first exploration of tissue- and cell-type-specific responses to heat stress in any model organism. Our findings will provide novel insights into autophagy regulation following HS in young and aged animals, contributing to therapeutic strategies to maintain proteostasis and promote healthy aging in humans.

Keywords: Aging, Autophagy, HLH-30/TFEB, Hormesis, Heat Shock, Auxin-inducible degradation (AID), single-cell RNA sequencing

887B Glial regulation of organismal ER stress resistance via XBP1 in *C.elegans*

Saebom Kwon[1], Emmett Griffin-Baldwin[1], Ashley E Frakes[1,21]Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, [2]National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health

The brain plays an important role in coordinating a whole-body response to stressors that threaten homeostasis such as pathogens, starvation, or aging. The unfolded protein response of the endoplasmic reticulum (*UPRER*) is activated in response to disrupted protein homeostasis in ER. Activation of the IRE1 branch of the *UPRER* leads to splicing of the transcription factor XBP-1 (XBP-1s) which induces transcription of chaperone and lipid metabolism genes to restore protein homeostasis. The ability to activate *UPRER* declines with age, and XBP-1 and its target genes are notably downregulated during aging in *C. elegans*, particularly in astrocyte-like glia. Astrocytes are a type of glia that interact with blood vessels and neurons in brain, allowing them to respond to metabolic changes and send signals to neurons or other peripheral tissues to restore homeostasis. We previously found that overexpression of *xbp-1s* in glia (*glial-xbp1sOE*) rescues age-onset loss of *UPRER*, increases resistance to chronic ER stress and extends lifespan. Therefore, we hypothesize that *xbp-1* in glia is necessary for animal survival from ER stress and loss of glial *xbp-1* animals are more vulnerable to ER stress. To elucidate this hypothesis, we generated worm strains with glial-specific deletion of *xbp-1* using FLP/FRT-based recombination (*glial-xbp1 KO*). Surprisingly, *glial-xbp1 KO* animals showed increased ER stress resistance similar to *glial-xbp1s OE* animals, while lifespan was normal. To determine how *glial-xbp1 KO* leads to increased ER stress resistance, we performed whole worm RNA-seq to identify differentially expressed transcripts. We observed increased expression of *UPRER* related genes in *glial-xbp1KO* animals. To elucidate the tissue-specific pattern of *UPRER* activation in *glial-xbp1 KO* animals, we used an *UPRER*-GFP reporter and found increased GFP in the intestine and spermatheca of *glial-xbp1 KO* animals. Moreover, because *xbp-1* also regulates lipid metabolism, we explored whether lipid levels were altered in *glial-xbp1 KO* animals using a fluorescent lipid droplet reporter strain. *Glial-xbp1 KO* animals showed increased lipid droplets in distal intestine. Currently, we are planning to conduct lipidome analysis and RNAi screens to identify what lipids are changed and if they are required for increased ER stress resistance. This study highlights how glia modulate XBP1 to regulate organismal ER stress resistance and lipid levels to survive in various stress conditions.

888B Circadian-mediated phenotypic heterogeneity drives immune variability among clonal individuals

Jonathan Lalsiamthara[1], Alejandro Aballay[1,21]Department of Genetics, UT MD Anderson Cancer Center, [2]Department of Microbiology and Molecular Genetics, UTHealth McGovern Medical School

The risk and severity of disease in individuals are influenced by factors such as genetics, epigenetics, nutrition, lifestyle, and environmental conditions. However, it remains uncertain whether inherent variations in immune responses against pathogen infection exist among clonal individuals within isogenic populations. To investigate this, we examined various physiological parameters at the individual level in isogenic, developmentally synchronized *Caenorhabditis elegans*. Our findings revealed that pathogen susceptibility within a clonal population is deterministic and can be anticipated using specific physiological markers. Individuals exhibiting high basal expression of the infection response gene *irg-5* were more susceptible to *Pseudomonas aeruginosa* infection. A genome-wide screen identified the MEIS homeobox protein UNC-62 as a regulator of *irg-5* basal expression, acting through ELT-2 and PMK-1 pathways. Further analysis revealed that maternal circadian rhythms contribute to immune activation heterogeneity among clonal offspring. In addition, RNA interference targeting the clock gene homolog *lin-42* eliminated circadian influences on the prevalence of offspring with elevated *irg-5* activation. These findings suggest that circadian-mediated phenotypic heterogeneity enables clonally reproducing organisms to utilize diurnal cues, generating heterogeneous progeny populations to enhance their chances of survival against pathogen infection.

889B The role of genetic sex in the regulation of environmental stress responses

Maria I Lazaro-Pena[1], Carlos A Diaz-Balzac[2], Douglas S Portman[31]Biomedical genetics, University of Rochester Medical Center, [2]Division of Endocrinology, Diabetes and Metabolism, University of Rochester Medical Center, [3]Biomedical Genetics, University of Rochester Medical Center

Environmental stress is inevitable for all living organisms, and prolonged exposure to it disrupts organismal physiology and behavior, ultimately affecting survival. For this reason, multicellular organisms are equipped with a variety of extracellular and intracellular mechanisms, enabling adaption and survival under diverse stress conditions. Despite previous efforts in the field to understand the genetic mechanism controlling the activation and regulation of different cellular stress-inducible response pathways, the factors that regulate the sensitivity and responsiveness of these pathways are not well understood. The nematode *C. elegans* has been extensively used as a model system to characterize stress response mechanisms at the cellular, tissue and organismal levels. However, nearly all these studies have been done using only hermaphrodites. In this study, we compare males' and hermaphrodites' resistance to two types of external stress, heat and osmotic stress. In both cases, we observed a sex-biased survival to stress, with males having a higher survival rate than hermaphrodites after exposure to heat and hypertonic stress. This sexually dimorphic resistance suggests that biological sex can regulate heat and osmotic stress response. To identify the tissue(s) in which sexual state regulates stress response, we used tissue-specific sex-reversal. Interestingly, feminization of the nervous system in males drastically reduced the survival rate of males when exposed to osmotic stress, but no significant reduction of male survival was observed when exposed to heat stress. These results suggest that the male nervous system potentiates osmotic stress response through a yet-unidentified mechanism. It also suggests that males' higher capacity to survive to heat and osmotic stress is regulated by different tissues, and potentially different mechanisms. Furthermore, we found that males have a higher induction of heat and osmotic stress-inducible genes compared to hermaphrodites, suggesting a higher activation of stress-responsive transcriptional programs in males. To better understand the mechanisms that bring about sex differences in stress response, we are performing RNAseq experiments comparing the transcriptome of both sexes after exposure to heat and osmotic stress. Identifying the links between biological sex and stress responses may identify previously unknown regulatory mechanisms that generate plasticity in animals' defense against, and susceptibility to, environmental stress.

890B Transgenerational regulation of longevity in *hsb-1* mutants of *C. elegans*

Phuong Thu Nguyen Le[1,2], Pi-Yu Ku[2], Chung-Yi Liang[3], Tsui-Ting Ching[2], Ao-Lin Hsu[4,51]Taiwan International Graduate Program in Molecular Medicine, National Yang Ming Chiao-Tung University and Academia Sinica, Taipei, Taiwan, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University, Taipei, Taiwan, [3]Division of Geriatric Medicine, University of Michigan, Ann Arbor, MI, USA, [4]Division of Geriatric Medicine, Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA, [5]Department of Molecular and Integrative Physiology, University of Michigan

Aging is an inevitable and irreversible decline in an organism's structural and functional integrity over time. Using *C. elegans*, we observed that the longevity phenotype in *hsb-1* mutants is gradually diminished over several generations. However, the genotype of *hsb-1* mutants is unchanged, suggesting that this loss of longevity might depend on epigenetic regulation. Intriguingly, we found that the long-lived phenotype of *hsb-1* mutants was rescued via outcrossing with wild-type N2 animals. Moreover, when short-lived *hsb-1* hermaphrodites were crossed with *hsb-1* males, the long-lived phenotype reappeared in the F1 progenies. These findings suggest that mating processes might reset the epigenetic marks in the zygote, restoring the long-lived phenotype. In this study, we identified that SCL-15, a male-specific protein in the seminal fluid, as a key trigger for epigenetic reprogramming in progenies of the short-lived *hsb-1* mutants. We found that SCL-15 expression is initiated upon male adulthood and specifically localized to the vas deferens, a critical conduit for sperm transport and ejaculation during mating. Furthermore, using transgenic SCL-15::GFP-tagged worms, we detected a prominent fluorescent signal within the uterus of mated hermaphrodites, indicating the transfer of SCL-15 from males to hermaphrodites during copulation. Additionally, our comparative gene expression analysis between the long-lived and short-lived *hsb-1* mutants identified two significantly upregulated Hedgehog-related genes, *wrt-1* and *wrt-10*, in the long-lived *hsb-1* mutants. Functional analysis confirmed that *wrt-1* and *wrt-10* are critical for *hsb-1* mutants to regulate the longevity phenotype. Knockdown of *wrt-1* or *wrt-10* reduced lifespan in long-lived *hsb-1* mutants, while their overexpression in short-lived *hsb-1* mutants extended lifespan. Taken together, our findings reveal a novel role of seminal fluid protein and Hedgehog-related genes in epigenetic reprogramming during sexual reproduction in animals.

Key words: *hsb-1*; longevity; mating; SCL-15; seminal fluid protein; Hedgehog; *wrt-1*; *wrt-10*.

891B A High Throughput Screen for Compounds That Impair Sleep

William J Lee[1], Marisa C Petticord[1], Joel Wooley[1], D'Juan Moreland[1], Zipporah Robinson[21]Neurology, University of Pennsylvania, [2]Spelman College

Sleepiness and fatigue are common symptoms during illness and may persist after the resolution of illness. There are no known treatments for the fatigue associated with sickness. Heightened sleep during sickness is observed in non-human animals, providing an opportunity to study the underlying mechanisms of sickness sleep and to find potential therapies. To gain insight into the neurochemistry of sickness-induced sleep and to discover therapeutic candidates, we performed a high throughput chemical screen using *Caenorhabditis elegans*. Among the 3,840 chemicals screened, 42 compounds strongly reduced sleep behavior, and we could replicate the results for 88% of the compounds we performed replication experiments for. These compounds can suppress sleep induced by virus infection, genotoxic injury, and proteotoxic injury, and they likely act downstream of activation of two sleep-promoting neurons, ALA and RIS. In addition to suppressing sleep in sickness (SIS), some of these compounds also suppress sleep in health, displaying a global suppression of sleep. We tested and rejected the hypothesis that these drugs act by increasing monoaminergic tone, providing evidence that these compounds act via a novel mechanism to block sleep.

892B Sexually dimorphic regulation of lipid metabolism by the hypothalamic-like AVK neuron *in C. elegans*

Chien-Po Liao, Hongzhu Ji, Oliver Hobert Biological Sciences, Columbia University in the City of New York

Sex-specific differences in lipid metabolism contribute to distinct disease risks and severities between the sexes. The hypothalamus plays a central role in lipid homeostasis by regulating energy expenditure and directly modulating lipid synthesis and storage in distal tissues. Anatomical differences in hypothalamic nuclei, along with sexually dimorphic outcomes following the ablation of specific hypothalamic neurons, highlight its crucial role in maintaining organismal homeostasis in a sex-dependent manner. However, the molecular mechanisms underlying this regulation remain largely unexplored.

We show that the *C. elegans* AVK interneuron is reminiscent of hypothalamic neurons at both the molecular and functional levels. We demonstrate that AVK regulates lipid metabolism in the intestine in a sex-specific manner. In *hlh-15* mutants, in which AVK is genetically ablated, we observe altered lipid droplet size and density in both sexes; however, only mutant males exhibit defective intestinal lipid storage. Furthermore, using CRISPR/Cas9-generated endogenous protein reporters for the Δ9 desaturases FAT-5, FAT-6, and FAT-7—key rate-limiting enzymes in monounsaturated fatty acid biosynthesis—we reveal sex-specific lipid regulatory pathways. Upon sexual maturation, males upregulate FAT-5, whereas hermaphrodites predominantly express FAT-6 and FAT-7. Loss of AVK disrupts this regulation, impairing male-specific FAT-5 upregulation while increasing FAT-7 expression in hermaphrodites.

We also show that AVK differentially regulates lifespan in a sex-dependent manner: in hermaphrodites, AVK negatively impacts longevity, possibly by inhibiting FAT-7 expression, whereas in males, AVK promotes lifespan, possibly by upregulating FAT-5. Together, our findings implicate AVK as a key neuronal regulator for sexually dimorphic intestinal lipid metabolism and lifespan, providing insights into how the hypothalamic-like neuron orchestrates the sex-specific lipid metabolic profile to maintain organismal homeostasis.

893B A Healthspan Screen in *C. elegans* Identifies Novel Compounds for Lifespan Extension

Gordon Lithgow, David Hall, Mustafa Sheikh Buck Inst

In order to facilitate the discovery of novel drugs and targets which modulate lifespan in the nematode *Caenorhabditis elegans*, we have developed a high-throughput chemical compound screening method which utilizes thermal stress survival at 37C as a positive predictor for assessment of potential pro-longevity effects in 20C worm survival assays. Utilizing a nucleic acid stain for identifying exclusively dead animals in wells, we are able to quickly assess survival conditions in liquid culture 96-well microtiter plate format across longitudinal time measurements in order to identify compounds which extend thermotolerance survival significantly longer than controls.

After screening two small compound libraries and then a separate validation process to confirm improved thermotolerance scores from candidate hit compounds, we then assess hits on conventional worm survival assays conducted on agar plates at 20C, of which a number of thermal-identified hits have been found to confer significant lifespan extension effects on wild-type animals. Through this method, we are able to quickly discover compounds with a high likelihood of conferring positive survival effects in the worm.

894B Low zinc homeostasis in *C. elegans*: A critical role for *zipt-2.3*

Hanwenheng Liu[1], Nicholas Dietrich[2], Samuel Herrera[1], Raquel Herrera[3], Adelita Mendoza[4], Kerry Kornfeld[1]1Developmental Biology, Washington University in St. Louis, [2]Developmental Biology, Kriya Therapeutics, [3]Washington University in St. Louis, [4]Biochemistry, University of Colorado, Boulder

Zinc is a critical element for protein structure and enzyme function. Thus, zinc deficiency can cause developmental and adult defects and diseases. In *C. elegans*, excess zinc is stored in lysosome-related organelles (LROs), causing structural remodeling that increases the volume of the expansion compartment. Zinc deficiency activates transcription of genes such as *zipt-2.3* through the low zinc activation (LZA) enhancer. *zipt-2.3* encodes a zinc importer that localizes to LRO membranes and releases stored zinc. How worms sense zinc deficiency and what transcription factor binds the LZA enhancer are unknonwn. To address these gaps in understanding, we performed a high throughput EMS mutagenesis screen for animals with defects in the zinc deficiency response. Using a *zipt-2.3* promoter-driven *mScarlet* reporter, we screened for red animals in normal conditions and non-red animals in zinc deficiency. We screened at least 400,000 genomes and recovered 18 mutant strains: 12 independent *zipt-2.3* mutants display a constitutive zinc deficiency response in normal conditions, and 6 mutants represent 3 additional complementation groups. These results indicate the crucial role of *zipt-2.3* in the zinc deficiency response. We are currently analyzing the *zipt-2.3* mutants as an allelic series, and we are using WGS to identify mutations in the other mutants.

To explore genes involved in the zinc deficiency response over time, we conducted an RNAseq time course experiment. We synchronized animals at the L4 stage, cultured them in zinc deficient conditions, and examined their gene expression profiles at 2, 4, 12, 16, and 24 hours. Through K means clustering analysis, we found 267 genes that are differentially expressed in zinc deficiency independent of development: 207 are induced and 60 are repressed. This provides novel insight into the global pattern of genes regulated by zinc deficiency. We are prioritizing genes based on the magnitude of expression change. We will to analyze their promoters to refine the understanding of the LZA enhancer sequence, and we will perform functional analyses through knockdowns and knockouts. These studies highlight the importance of *zipt-2.3* in the zinc deficiency response and identify new gene candidates involved in zinc deficiency homeostasis.

895B Non-Visual Light Sensing Enhances Behavioral Memory and Drives Gene Expression in *C. elegans*

Junqiang Liu[1], Zhijian Ji[2], Bingying Wang[2], Rashmi Chandra[21]Cardiovascular Research Institute, University of California, San Francisco, [2]University of California, San Francisco

Visible light is known to regulate various physiological processes, such as circadian rhythms, hormone secretion, and metabolic functions. However, the mechanisms by which animals detect and respond to light independently of the visual system remain poorly understood. Here, we uncover a previously undescribed light-induced transcriptional pathway that modulates behavioral plasticity in *C. elegans*, a roundworm without eyes. We demonstrate that ambient visible light or controlled-intensity visible-spectrum LED activates an effector gene *cyp-14A5* in non-neuronal tissues through the bZIP transcription factors ZIP-2 and CEBP-2. Light induction of *cyp-14A5* is more prominent at shorter wavelengths but is independent of the known blue light receptors LITE-1 and GUR-3 in *C. elegans*. This bZIP-dependent genetic pathway in non-neuronal tissues enhances behavioral adaptability and olfactory memory, suggesting a body-brain communication axis. Furthermore, we use the light-responsive *cyp-14A5* promoter to drive ectopic gene expression, causing synthetic light-induced sleep and rapid aging phenotypes in *C. elegans*. These findings advance our understanding of light-responsive mechanisms outside the visual system and offer a new genetic tool for visible light-inducible gene expression in non-neuronal tissues.

896B Development of sectioning methods to correlate the lipid molecular information with anatomy in *C. elegans*

Sara Mandic[1], Bryn Flinders[2], Michiel Vandenbosch[2], Akane Ohta[3], Atsushi Kuhara[3], Ron M. A. Heeren[2], Masazumi Fujiwara[11]Okayama University, [2]Maastricht University, [3]Konan University

Due to their well-established biological traits, C. elegans have become a popular choice for a variety of studies in different fields of biological research, such as developmental biology, neurology and genetics. As a result of these scientific efforts, many techniques have been applied in their research. In these projects, one of the commonly mentioned advantages of C. elegans as a model organism is their small size, making the maintenance easier and more cost-effective. Despite this, their size can also be disadvantageous for handling and sample preparation. An upcoming technique where the issue has been more noticeable is mass spectrometry imaging (MSI). This technique can show the spatial distribution of a wide range of analytes and can be used in multi-modal approaches which could be beneficial for C. elegans research as well. However, the sectioning required for MSI analysis has proven challenging for small samples such as C. elegans. In our work, we present a newly developed workflow that not only enables MSI analysis and different staining methods, but also retains the internal structures of the nematodes throughout consecutive sections. This finally allows for subsequent 3D reconstruction of both the optical images and those obtained from other modalities. Using these corresponding 3D reconstructions, we were able to visualize and follow the distributions of molecules through the nematode.

We used a commercial microfluidic device for the exact alignment of nematodes. The sample block was created using a mixture of gelatine and carboxymethylcellulose to encapsulate the C. elegans in the center of the block. The frozen block was later sectioned using a cryotome and the sections were collected on ITO glass slides. They were coated with a matrix and analyzed using two MS-based systems for visualization and identification. The results showed a variety of masses generally corresponding to lipids due to the selected matrix. For example, a mass present in all samples at m/z 466.3303 was putatively identified as sophosphatidylethanolamine (LPE 18:0) in the [M- H]- form, via the LIPID MAPS database. Additionally, when overlayed together, different lipid species showed complementary distributions, allowing for the determination of specific locations of these lipids within the analyzed nematodes.

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897B Tissue-specific roles of SKN-1 in neuronal activity and stress resistance

Hrishikesh Mane, Adriana San Miguel Chemical and Biomolecular Engineering, North Carolina State University

Reactive Oxygen Species (ROS), produced during mitochondrial respiration, can oxidize biomolecules, disrupting cellular function. Cells maintain redox homeostasis by deploying antioxidant enzymes to scavenge the ROS produced. Cellular damage caused by oxidative stress, where ROS production exceeds ROS scavenging, is said to be involved in the progression of various neurodegenerative diseases and theorized to be a cause of aging. Neurons being energy intensive, are known to produce high amounts of ROS. The most prominent pathway dealing with oxidative stress in *C. elegans* is the *skn-1* pathway. When *skn-1* is active, it is shown to increase oxidative stress resistance and lower neuronal activity in worms. However, it is not entirely clear how *skn-1* mediates neuronal activity and whether this effect is independent of organismal activation of antioxidant transcriptional pathways.

In this work, we first tested if there is a relationship between the level of *skn-1* activity and the subsequent reduction in neuronal activity. We subjected *C. elegans* to different concentrations of juglone and measured *skn-1* activity through *gst*-4 expression and neuronal activity using the aldicarb paralysis assay. Our results suggest that the reduction in neuronal activity is proportional to the level of *skn-1* activity. It was also interesting to see that the *skn-1* activity peaks at a certain concentration and then drops at even higher concentrations. Next, we looked at publicly available RNA-seq data of genes downstream of *skn-1* that act in the neuromuscular junction. An RNAi screen to identify the genes involved in altering neuronal activity revealed 4 genes that alter neuronal activity downstream of *skn-1*. Available literature suggests that *C. elegans* mutations with higher neuronal activity (e.g., *spr-3, spr-4, slo-1* etc.) generally have lower oxidative stress resistance. Therefore, we tested for the oxidative stress resistance provided by these downstream genes and observed that silencing these genes lowers oxidative stress resistance. Next, we tested the tissue specificity of *skn-1* activity in modulating neuronal activity. Silencing *skn-1* in neuronal and non-neuronal tissues using RNAi revealed that *skn-1* reduces neuronal activity through neurons but provides oxidative stress resistance through non-neuronal tissues.

Thus, our results suggest that *skn-1* acts independently to modulate neuronal activity and oxidative stress resistance via multiple genes and tissues in *C. elegans.*

898B Investigating the Potential of Probiotocs in the promotion of Muscle Health in Aging: A *C. elegans* Model study

Shibi Mathew, Noémie Auclair-Ouellet, Stéphane Bronner, Sylvie Binda Rosell Institute for Microbiome and Probiotics, Montreal, QC, Canada

Aging is related to loss of muscle mass, strength, and function, which can significantly impact quality of life in older adults. Recently, the gut-muscle axis has emerged as a useful model to explain bidirectional connections between the gut microbiota and the musculoskeletal system. Findings from related research suggest that inflammation and alterations in gut microbiota metabolic activity may play a critical role in muscle wasting. Probiotics have emerged as a promising therapeutic strategy to promote muscle health in aging, potentially enhancing muscle strength, increasing muscle mass, and improving overall functionality. Using Caenorhabditis elegans (C. elegans) as a model organism, this study evaluated the effects of probiotic diets on lifespan and various indicators of muscle health and function. Wild-type N2 and transgenic RW1596 worms (myo-3(st386) V; stEx30-) were fed one of three probiotic strains from the Bifidobacterium (B1, B2) or Lactobacillus (L) genus, a blend of these three strains, or a control diet of Escherichia coli OP50. The study assessed worms' longevity, mobility (measuring speed and distance), muscle stamina through exercise, and neuromuscular function using a burrowing test. Compared to the control diet, both B1 and the probiotic blend significantly extended lifespan in wild-type N2 worms. The probiotic blend also improved mobility, muscle stamina, and neuromuscular health in both wild-type N2 and transgenic RW1596 worms. These findings support the potential of probiotics as a strategy to enhance muscle function and mitigate age-related declines in muscle mass and strength. Future studies that explore the molecular mechanisms underlying these effects will help further elucidate the gut-muscle axis and help develop targeted strategies to support muscle health in aging populations.

899B Mitochondrial ROS spikes as a somnogenic signal from metabolic tissues

Pearson McIntire[1], David Raizen[2,3], Alexander Van der Linden[1]1Biology, University of Nevada Reno, [2]Neurology, University of Pennsylvania, [3]Chronobiology and Sleep Institute

Despite evidence of interactions between metabolism and sleep, the mechanisms underlying these interactions are not clear. KIN-29/SIK is an important regulator in sleep-metabolism interactions (Grubbs *et al.*, 2020). Mutants lacking *kin-29* function exhibit high triglyceride levels as well as reduced sleep. Genetically inducing lipolysis corrects both the adipose and sleepless phenotypes of *kin-29* mutants. To identify pathways mediated by KIN-29, we performed both proteomic and transcriptomic analyses of young adult *kin-29* mutants. Many of the identified proteins and transcripts with increased abundance were encoded by genes involved in the mitigation of reactive oxygen species (ROS). Previous work in flies and mammals showed that intestinal ROS was the causative agent in lethal sleep deprivation (Vaccaro *et al.*, 2020); however, the purpose of ROS in healthy sleep responses remains unclear. Our multi-omic study of *kin-29* mutants combined with these past findings led us to hypothesize that KIN-29 uses intestinal mitochondrial ROS accumulation from fat catabolism as a sleep pressure signal in the metabolic regulation of sleep. In support of this hypothesis, we found that intestinal mitochondrial ROS as measured by the HyPer7 ROS sensor, spikes during stress-induced sleep (SIS) and dissipates as wild-type animals awaken. In contrast, *kin-29* mutants have a decreased mitochondrial ROS response to somnogenic stimuli matching their reduced sleep phenotype. To further assess whether increases in ROS effect sleep, we screened *sod* mutants for SIS phenotypes. Double mutants for the mitochondrial *sod*s (including *sod-3*) slept more than wild-type animals. This effect becomes strongest in quintuple mutants that delete all five *sod*s. The increased SIS phenotype of the *sod* quintuple mutant was mitigated by reducing the UV irradiation dosage, suggesting that their sleep phenotype is due to an impaired ability to mitigate ROS. Cytosolic *sod* mutants did not show an SIS phenotype and cytosolic ROS levels did not significantly increase during SIS, suggesting that cytosolic ROS is not necessary to promote sleep. Currently, we are investigating the role of mitochondrial ROS in the metabolic regulation of sleep by testing the effect of optogenetic induction of ROS at different complexes of the electron transport chain in *kin-29* mutants.

900B The master virulence regulator BvgS in *Bordetella atropi* is required for invasion of intestinal cells

Serena J Meadows-Graves[1], Tuan D Tran[2], Robert J Luallen[21]Biology, San Diego State University, [2]San Diego State University

Bordetella atropi is an intracellular pathogen that infects the intestine of the host nematode *Oscheius tipulae* via a novel mechanism of cell-to-cell spreading. This bacterium enters the intestinal cell and undergoes a morphological change where it divides without septation to spread to adjacent cells. This mechanism has been shown to be regulated by the glucolipid pathway, whereby excess UDP-glucose serves as an indicator for nutrient-rich conditions inside the cell, temporarily inhibiting FtsZ ring formation.

To discover which virulence systems were required for host cell invasion, we systematically knocked out the type secretion systems in *B. atropi.* We found that intestinal cell invasion by this bacterium requires the type III secretion system (T3SS), a virulence mechanism which allows Gram-negative pathogens to inject effector proteins into the host cytoplasm. Knockout of structural components of the T3SS complex in *B. atropi* resulted in bacterial accumulation in the gut lumen and an inability to invade host cells in vivo, which could be rescued by complementation. We conducted dual RNAseq and found that the T3SS is highly induced in vivo, along with putative effector genes. Interestingly, we found that a master virulence regulator (*bvgS*) was also induced in vivo, but not in a T3SS mutant. Virulence in pathogenic Bordetellae is predominantly controlled by the highly conserved BvgAS master virulence regulator, which is composed of a sensor kinase BvgS and response regulator BvgA. This two-component systems allows the pathogen to switch between virulent and avirulent modes and control the transcription of genes which either promote or repress virulence. We knocked out the sensor kinase BvgS in *B. atropi* and found the bacteria failed to invade host intestinal cells and filament, recapitulating the T3SS knockout strains. Altogether, our data indicates that BvgAS likely regulates T3SS-mediated invasion in *B. atropi*, suggesting that the role of this virulence regulator is conserved in different Bordetellae including those that infect nematodes and mammals.

901B Metabolic profiling of sickness-induced quiescence in *C. elegans*

Salvador Mendez Fernandez[1], Pearson Mcintire[2,2], Emmanuel Adeyemi[2], David M Raizen[3], Alexander M van der Linden[21]University of Nevada, Reno, [2]Biology, University of Nevada, Reno, [3]University of Pennsylvania

Sleep disturbances, reduced activity, and fatigue are common symptoms of viral infections such as Covid19, but molecular mechanisms responsible for the virus-induced sleep are unclear. Viral infection by the Orsay-virus can induce quiescence in *C. elegans* (Iannacone et al. 2024), a state resembling sickness sleep observed in other animals. Orsay-virus infection is associated with decreased ATP levels, with a more pronounced decrease in sleep-defective mutants. To further explore the metabolic consequences of infection ± sleep, we performed a global untargeted metabolic profiling experiment. Viral infections were performed in a *rde-1*-deficient mutant background and we used mutants in the gene *ceh-17* to impair sickness sleep. Consistent with previous findings, infected animals exhibited an increased viral load as determined by RT-qPCR. Principle component analysis of the metabolites clearly differentiated experimental groups. Our approach identified a total of 1,003 metabolites, of which 959 were known metabolites ($P<0.05$). A two-way ANOVA revealed that the interaction between sleep and infection altered the abundance of metabolites for fatty acids and carnitines. Infection significantly altered the abundance of metabolites involved in the biogenesis of peptidoglycans. Sleep altered abundance of metabolites for glutathione, phospholipids, nicotinamides and energetics. We suggest that these pathways may contribute to Orsay-induced quiescence and/or to the metabolic consequences of quiescence. We are testing mutants for genes controlling these metabolic pathways to determine their role in Orsay-induced quiescence.

902B Post-translational oxidation on AMPK regulates lifespan and stress resistance in *C. elegans*

Yalin Liu[1], Jin Meng[1,21]Capital Medical University, [2]Chinese Institutes for Medical Research

The evolutionarily-conserved AMP-activated protein kinase (AMPK) serves as a central regulator of cell growth, metabolism, mitochondrial function, and autophagy. It is activated upon low nutrient levels or energy stress. *C. elegans* AMPK alpha subunit AAK-2 mediates pro-longevity interventions such as dietary restriction, mTORC1 suppression, and reduced insulin/insulin-like growth factor (IGF-1) signaling. AAK-2 activation alone is also sufficient to extend lifespan, whereas loss of AAK-2 shortens lifespan. We have previously shown that AAK-2 contains a conserved cysteine C245 in the kinase domain that can be modified by low-dose of H2O2, but the physiological relevance of this post-translational modification is not clear. Here we have generated mutant worms that carry a cysteine-to-serine mutation in the endogenous *aak-2* gene using the CRISPR/Cas9 gene editing tool. We have found that replacement of C245 into an oxidation-inert serine partially impairs longevity arising from mTORC1 inhibition or elevated reactive oxygen species (ROS). In addition, the oxidation-deficient mutation reduces stress resistance of old worms, suggesting that oxidation at this cysteine is needed for AMPK activation under stressed conditions. Together, our findings indicate that AAK-2 acts as a ROS sensor, and oxidation at a conserved cysteine plays an important role in the regulation of lifespan and stress resistance by AMPK.

903B Dauer formation enhances fertility under heat stress in *Caenorhabditis* nematodes

Stephen E Miceli[1], Lisa N Petrella[21]Marquette University, [2]Department of Biological Sciences, Marquette University

Caenorhabditis elegans is a well-established model organism for studying fertility. In the wild, nematodes frequently enter the dauer stage, a stress-resistant developmental arrest that enhances survival under unfavorable environmental conditions such as high population density and food scarcity. While dauer formation is known to extend nematode survival, its role in protecting fertility after environmental stress is unclear. We are studying whether dauer formation impacts fertility following exposure to heat stress in two nematode species, C. elegans (N2) and C. briggsae (QX1410). Specifically, we compare the fertility levels of individuals that underwent dauer formation with those that developed continuously without entering dauer. By using an established protocol, we were able to induce and isolate dauer larvae starvation conditions and selectively eliminate any non-dauers using the dauer's unique morphology of a closed mouth. Dauer larvae were placed on food until they reached the post dauer L4 stage. Post-dauer and non-dauer L4s were upshifted to moderate stress temperatures for the species: 27°C for C. elegans (N2) and 31°C for C. briggsae (QX1410). Post-dauer and non-dauer fertility was assessed by monitoring for F1 and F2 progeny production. Our early results suggest that dauer formation plays a protective role in maintaining fertility under heat stress conditions. Nematodes that had gone through dauer formation showed higher fertility rates following heat stress compared to their non-dauer counterparts, supporting the hypothesis that dauer formation enhances stress resistance. These findings provide insights into how Caenorhabditis and other organisms balance survival and reproductive success under environmental stresses such as temperature fluctuations.

904B Determining how neuronal stress drives progressive distal tissue dysfunction during aging

Jason W Miklas[1], Mikaela Wilson[2], Sasha Tsenter[2], Guillaume Bogaerts[2], Anne Brunet[21]Genetics, Stanford University, [2]Stanford University

Aging is associated with a decreased resilience to stress stimuli and an increased onset of many age-related diseases. However, the interactions between age and stress stimuli are not well understood. In neurodegenerative diseases, neuronal stress progressively worsens with age. Intriguingly, distal tissues can also rapidly deteriorate in an age-dependent manner in some neurodegenerative diseases. For example, in Parkinson's disease, distal tissue pathologies occur in the intestine, and the crosstalk between neurons and intestine is complex. There is an urgent need to identify the set of secreted proteins that drive distal tissue dysfunction in response to the combination of old age and chronic neuronal stress, as this could lead to new interventions. To model age-progressive chronic neuronal stress, we generated *C. elegans* strains that express a single copy of alpha-synuclein (SNCA), a disease-associated protein in Parkinson's disease, only in neurons. We find that *C. elegans* expressing alpha-synuclein (wild-type or the familial A53T point mutant) in neurons exhibit a decline in neuron morphology with age. Interestingly, we showed that this type of neuronal stress results in accelerating intestine dysfunction. Individuals expressing alpha-synuclein (wild-type or the familial A53T point mutant) exhibit intestine barrier dysfunction and defecation defects with age. To quantitatively characterize a tissue's secretome across age, we previously developed a tissue- and compartment-specific protein labeling tool by localizing the biotinylating protein TurboID to the intestine endoplasmic reticulum of *C. elegans*. We have shown that these proximity labeling tools, coupled to quantitative proteomics, are powerful to identify previously uncharacterized secreted proteins in the intestine, and we have now built new tools to probe the neuron secretome in *C. elegans*. We have generated strains to characterize the intestine and neuron secretomes in the context of the neuronal alpha-synuclein (wild-type or the familial A53T point mutant). By leveraging our suite of secretome *C. elegans* strains, we are well-suited to understand how aging and neuronal stress perturb the neuron and intestine secretomes, with the goal of identifying key conserved secreted proteins that can be leveraged to restore distal tissue dysfunction during aging and age-related diseases.

905B *gsk-3* and its protective role in proteostasis within *C. elegans*

Callie Millette, Joslyn Mills Biology, Bridgewater State University

Huntington's disease (HD) is a devastating neurodegenerative disorder caused by an expansion of CAG repeats in the HTT gene, leading to the production of the mutant huntingtin (mHTT) protein. This protein aggregates, disrupting cellular function and causing neuronal death. To investigate genetic modifiers of mHTT aggregation, we utilized the EAK103 strain of *Caenorhabditis elegans*, which expresses mHTT tagged with yellow fluorescent protein (YFP) in body wall muscles. In a reverse genetic screen of RNAi against 60 candidate genes, *gsk-3* was identified to cause an increase in mHTT aggregation when knocked down. *gsk-3* encodes glycogen synthase kinase-3, a serine/threonine kinase implicated in cellular homeostasis, proteostasis, and neurodegenerative diseases.

This study hypothesized that the exacerbation of mHTT aggregation due to *gsk-3* knockdown will impair health, lifespan, and proteostasis while increasing susceptibility to addiction-like behaviors. Using EAK103, we examined the impact of *gsk-3* knockdown on lifespan, fitness, learning, memory, and nicotine addiction traits. We revealed that *gsk-3* knockdown decreased lifespan and thrashing activity compared to controls, indicating impaired physical health and neuromuscular coordination. Behavioral assays further demonstrated cognitive deficits, such as impaired learning, memory, and addiction propensity in *gsk-3* knockdown nematodes.

These findings will highlight the critical role of *gsk-3* in maintaining cellular health and its influence on addiction vulnerability in HD models. They suggest that *gsk-3* dysregulation may exacerbate neurodegenerative processes and heighten susceptibility to addictive substances. This research provides novel insights into the interplay between neurodegeneration and addiction, paving the way for potential therapeutic strategies to address HD and related conditions in humans.

906B Exploring the disruption of copper homeostasis by CL-5, a novel anthelmintic

Jennifer Miskowski Biology, Univ Wisconsin, La Crosse

CL-5 is a novel compound that possesses anthelmintic activity in *Caenorhabditis elegans*. CL-5 was generated from a parent compound isolated from the sweet fern plant, *Comptonia peregrina*, and it is a stilbene that is structurally like resveratrol. Although resveratrol exhibits antioxidant activity, CL-5 causes developmental delays, paralysis, and death in worms which is associated with elevated oxidative stress. The DAF-16 pathway is triggered in CL-5-treated animals, and qRT-PCR was used to measure gene expression of known DAF-16 targets. Most notably, *mtl-1* was dramatically upregulated in response to CL-5. MTL-1 is a metallothionein which acts to bind metal ions and maintain homeostasis. Disruption of metal ion levels is linked to oxidative stress and several disease states, and initial evidence suggests that CL-5 specifically affects copper levels. The importance of copper homeostasis is illustrated by the ramifications of its disruption; copper deficiency causes Menkes disease while copper excess leads to Wilson disease, both of which are fatal if untreated. Two different mutants defective in copper transport, *cua-1* and *cuc-1*, showed heightened sensitivity to drug. This sensitivity was exacerbated if the plates contained a sub-toxic level of CL-5 along with low levels of added copper, indicating an additive effect. Furthermore, worms cultured on plates that contained CL-5 and BCS, a chemical that specifically sequesters copper to minimize its toxicity, were less affected. The expression levels of *cua-1* and *cuc-1* were measured using qRT-PCR; both genes were upregulated ~2-fold in CL-5 exposed worms. CUA-1 is the worm ATP7A/B ortholog, which is a copper transporter that functions in the intestine to ensure copper homeostasis. When copper levels are high, CUA-1 is localized to gut granules that store excess copper so that toxic levels are not distributed throughout the organism, but under normal or low copper levels, CUA-1 is primarily found in the plasma membrane of intestinal cells. Experiments are underway to test mutant strains that are defective in gut granule biogenesis to determine if they are more sensitive to CL-5. In addition, the localization pattern of CUA-1 within the *C. elegans* intestine upon CL-5 treatment will be investigated. Lastly, although 100% of *C. elegans* adults exposed to high levels of CL-5 die, ~40% of their progeny survive and experience developmental delays. Continuous exposure to drug renders these animals resistant, although that effect is reversed if drug is removed. Gene expression changes in survivors is being explored and compared to that of the parental generation.

907B Systems Biology using *C. elegans*: The influence of genetic variations on drug response

Fathima S. Mohammed Refai[1,2], Hala Fahs[3], Gennaro Esposito[4], Yasmine Moussa[4], Suma Gopinadhan[4], Antony Page[5], Erik Andersen[6], Kristin C Gunsalus[3,4], Fabio Piano[3,41]Science, New York University Abu Dhabi, [2]Università di Udine, [3]New York University, [4]New York University Abu Dhabi, [5]University of Glasgow, [6]Johns Hopkins University

Biologically active compounds are the foundation of most therapeutics and serve as essential tools for investigating cellular processes. We developed a high-throughput, high-content robotic screening platform to identify bioactive small molecules and their targets, with a focus on discovering novel disease treatments and broad-spectrum anthelmintics. We screened 50,000 compounds for potential broad anthelmintic properties and counter-screened for molecules that are non-toxic in a human cell line. We identified 20 potent hits with activity in anthelmintic-resistant strains, indicating that they act through novel mechanisms of action. We are studying how natural genetic diversity influences the response to these bioactive molecules using the Caenorhabditis Natural Diversity Resource (CaeNDR). We plan to analyze responses across genetically distinct strains to a panel of novel potential anthelmintics identified in our screen. By integrating genome-wide association (GWA) mapping, forward and reverse genetics, and metabolomics, we aim to uncover the molecular basis of drug sensitivity and resistance, identifying both target-specific effects and indirect responses. These insights will deepen our understanding of the pharmacogenomics of anthelmintic activity, enabling us to make significant progress in tackling anthelmintic drug resistance.

908B Regulation of poly-glutamine protein aggregation and proteostasis by the ubiquitin-like protein UFM-1

Samantha Moores[1], Betty Ortiz Bido[2], Peter Juo[31]DMCB, Tufts University Graduate School of Biomedical Sciences, [2]Rockefeller University, [3]Tufts University Graduate School of Biomedical Sciences

The ubiquitin-like molecule (UBL) Ubiquitin-Fold Modifier-1 (UFM-1) has been implicated in protein quality control and the stress response; however, its physiological functions are still poorly understood. Conjugation of UFM-1 (Ufmylation) to substrates is mediated by an enzyme cascade consisting of an E1 activating enzyme (*uba-5*), an E2 conjugating enzyme *(ufc-1*) and an E3 ligase (*ufl-1*). Models of poly-glutamine (polyQ) expansion diseases, like Huntington's Disease, have been developed in *C. elegans* and can be used to monitor perturbations in proteostasis (Faber et al. 1999; Morley et al. 2002; Nollen et al 2004). These diseases are caused by CAG repeats that encode expanded polyQ tracts that are prone to misfolding and aggregation. I found that loss-of-function mutations in *uba-5*, *ufc-1 or ufl-1* lead to increased protein aggregation of a muscle-expressed GFP-tagged polyQ protein with 40 glutamines, suggesting that the UFM-1 pathway may act to prevent protein aggregation. Interestingly, loss of *ufbp-1*, an ER-anchored protein that recruits UFM-1 pathway components to the ER surface, also results in increased polyQ protein aggregation, suggesting that the UFM-1 pathway may act at the ER to regulate the cytosolic aggregation of polyQ proteins. Further, our tissue specific rescue experiments suggest that the UFM-1 pathway acts in neurons and muscle to regulate polyQ aggregation in the muscle. These findings indicate that the UFM-1 pathway can act cell autonomously and cell non-autonomously to regulate proteostasis in the muscle, and that there is a neuron-to-muscle signal that regulates proteostasis. The UFM-1 pathway has been shown to regulate the ER Unfolded Protein Response (UPRER) (Hertel et al. 2013; Walczak et al. 2018), which is responsible for alleviating ER stress from misfolded proteins and restoring proteostasis. I have found that disruption of the UFM-1 pathway leads to increased survival under acute ER stress. Future work will focus on further investigating the relationship between the UFM-1 pathway and the UPRER and the mechanism by which UFM-1 pathway activation at the ER regulates cytosolic aggregation of polyQ proteins.

909B Mechanical Force Triggers Extrusion of Large Vesicles from *Caenorhabditis elegans* Mechanosensory Neurons

Andrés A Morera, Maria Ivanova, Guoqiang Wang, Edward Chuang, Monica Driscoll Molecular Biology and Biochemistry, Rutgers University

C. elegans neurons can form large diameter (>4µm) extracellular vesicles called "exophers". Exophers are laden with cytoplasmic contents and are hypothesized to constitute a mechanism of eliminating toxic protein aggregates and dysfunctional organelles to maintain cell function under conditions of proteotoxic and oxidative stress. The capacity for exopher production may be a conserved phenomenon across species with applicability to human disease, as exophers have also recently been described in mammalian models.

Recent findings from our lab have suggested that exophers may form due to extrinsic mechanical forces acting upon the cell (Wang *et al.* 2024). Under normal circumstances, exophers from mechanosensory neurons in adult hermaphrodite worms require the presence of eggs in the uterus, and the proximity of these neurons to eggs highly correlates with their ability to produce exophers. We have taken studies on pressure induction of exophers a step further using a microfluidics device with pneumatic actuators to squeeze worms at locations adjacent to mechanosensory neurons (Nekimken *et al.* 2017). With this, we have been able to trigger exopher release from these neurons under conditions where exophers otherwise do not form, namely in infertile adult and larval worms. Together, our findings suggest that eggs may contribute to neuronal exopher formation through the forces they exert upon these neurons.

Work planned for the immediate future will more directly characterize the relationship between exopher formation, the physical forces experienced by the neuron, and factors that alter the frequency of exopher production, such as age, the presence or absence of eggs, and disruption of proteostasis. To this end, we are employing a beta spectrin-based FRET biosensor (Krieg *et al.* 2014) to directly measure the state of tension or compression of the neuronal cytoskeleton under exopher-permissive and exopher-inhibitive conditions.

Beta spectrin-based FRET biosensor constructs and strains were graciously provided by the lab of Dr. Miriam Goodman. The microfluidics device used in the present work was a gift from the laboratory of Dr. Michael Krieg.

910B A battle of the TFs: a multi-transcriptional crosstalk regulates oxidative stress resistance, lipid synthesis and longevity

Jorge I. Castillo-Quan[1,2], Aiden McCarty[3], Ugne Kurdeikaite[1,3], Katherine Gilmore[3], Arlette Cabral[3], Justin Mejia[3], Maria La Terza[3], Julia Barrett[3], Emma Johnson[3], Allison Carroll[3], T. Keith Blackwell[1,2], Natalie Moroz[31]Joslin Diabetes Center, [2]Genetics, Harvard Medical School, [3]Biology, Emmanuel College

Aging is an inevitable process experienced by all organisms and characterized by a gradual decline in function. Aging increases the risk of age-associated diseases (AAD) such as cardiovascular disease and Alzheimer's disease. It is suggested that an accumulation of reactive oxygen species contributes to AAD pathology while increased xenobiotic detoxification is found in many long-lived mutants across model organisms. In *C. elegans*, the transcription factor SKN-1C, an ortholog to the NF-E2-related transcription factor (Nrf2) is a master regulator of xenobiotic detoxification genes and the antioxidant response. Using a strain with increased SKN-1C/Nrf2 expression, we conducted a genome-wide RNAi screen to identify novel upstream regulators of SKN-1C. Among the hits we identified another transcription factor, Krüppel-like factor-1 (KLF-1), which has been shown to regulate Phase I detoxification genes and lipid homeostasis. We found that KLF-1 is required for the SKN-1C antioxidant response, placing it upstream of SKN-1C. We looked at KLF-2, another KLF ortholog, and found it required for oxidative stress resistance, but not by regulating SKN-1C. Under steady state conditions and in mutants displaying enhanced lipid accumulation, we found knockdown of *klf-1* reduced triglycerides while knockdown of *klf-2* did the opposite. This was independent of *skn-1a*, a less studied SKN-1 isoform found to mediate fatty acid beta-oxidation. Interestingly, we found that RNAi against both *klf-1* and *klf-2* eliminated any change in lipid homeostasis suggesting the two transcription factors may regulate one another or act on similar downstream targets. Lastly and importantly, KLF-1 completely suppressed lifespan extension mediated by increased SKN-1 activity, while KLF-2 only had a partial effect. These findings suggest that the role of KLF-1 in detoxification, upstream of SKN-1C/Nrf2, has a consequential effect on longevity, possibly by reducing lipid oxidation and its inherent toxicity. We have thus placed KLF-1 and SKN-1C/Nrf2 in a regulatory network that tightly governs the antioxidant response, KLF-1 and KLF-2 regulating lipid homeostasis in opposition to one another, and KLF-1 likely in a multifaceted role to reduce lipid peroxidation, in organismal aging. This multi-transcription factor crosstalk may be important in diseases where ROS contributes to pathology or lipid homeostasis is lost.

911B TORC2/RICTOR regulates an interspecies crosstalk that influences longevity through a novel mitochondrial axis

Simran Motwani, Somya Bhandari Molecular Aging Laboratory, National Institute of Immunology

Normal life history traits of an organism are shaped by its ability to adapt to the dynamic environmental challenges within its niche, including fluctuations in food availability and quality. This adaptive capacity is maintained by intricate diet-gene interactions, many of which remain poorly understood. Our study demonstrates that the conserved mTORC2 catalytic subunit, RICTOR, plays a critical role in maintaining adaptability to variations in the bacterial diet of *C. elegans*. When fed a nutrient-rich diet, the *rict-1* mutant shows enhanced tolerance to osmotic stress and an extended lifespan. These phenotypic changes depend on appropriate mitochondrial function and metabolic outputs. Notably, the enhanced phenotypes of the *rict-1* mutant are modulated by bacterially derived metabolites. Our findings reveal a novel mechanism by which RICTOR/TORC2 prevents bacterially derived metabolites from impacting host cellular functions and lifespan.

912B High-content longitudinal imaging of *C. elegans* for biological age prediction

Alexandre Vaudano[1], Lazar Stojković[1], Matteo Cornaglia[1], Florence Solari[2], Laurent Mouchiroud[1]1Nagi Bioscience SA, EPFL Innovation Park, [2]Université Claude Bernard Lyon 1 Institut NeuroMyoGène

Understanding how an organism remains healthy later in life is an important goal of aging research. Transcriptomic clocks are refined tools that enable prediction of biological age based on molecular data with remarkable accuracy in multiple organisms, including *C. elegans*. Yet, in *C. elegans*, these clocks require the sacrifice of the worms and thus hamper longitudinal measurements.

Here, we present a non-invasive phenotypic aging clock for *C. elegans* that enables the quantification of biological age at the individual worm level throughout its lifespan.

Using the automated SydLab platform, we tracked close to 4500 worms at 6h intervals throughout their whole lifespan, generating a comprehensive dataset of 29 phenotypic features related to aging, such as motility, morphology, and reproductive parameters. These data were used to develop an aging clock able to predict the biological age of individual worms with a median R2 of 0.84 (0.79-0.86; interquartile-range, IQR). While accurately predicting biological age at single timepoints, the clock also provides an unprecedented vision of aging trajectories at the individual level.

By day 12, biological age predictions from our aging clock distinguish interventions that extend lifespan from those with no effect with 80% accuracy and 91% specificity. This is accomplished in less than half the time required for a full lifespan assay and using as few as 40 worms per condition. Moreover, when performing a complete lifespan assay, our approach can be combined with standard survival measurements, helping to dissociate healthspan from lifespan effects.

To validate our approach, we conducted a preliminary study with several mutant strains (the long-lived *slcf-1*(*tm2258*) and the short-lived *daf-18*(*e1375*)) comparing a transcriptomic clock (BiT age) with our phenotypic clock. While the two clocks showed a strong agreement in their predictions for slcf-1 and daf-18, the double mutant *daf-18*(*e1375*);*slcf-1*(*tm2258*) revealed discrepancies between transcriptomic age and phenotypic age. This may suggest additional complexities in the coupling between transcriptional changes and functional biomarkers in worm aging.

Our results highlight the value of real-time functional phenotyping for aging research in *C. elegans*, complementing existing molecular-based methods. Thanks to its image-based nature, our approach is inherently scalable and adaptable to multiple experimental designs, offering potential for early drug screening and investigations that require repeated measurements of the same animals. Future work will aim on integrating tissue-specific phenotypes and fluorescence data and constructing organ-specific clocks, ultimately bridging the gaps between biological scales.

913B Bringing *in vivo* data at the *in vitro* scale: A fully automated high-data throughput platform for *C.elegans* phenotypic screening

Marie Pierron, Fabien Tache, Alain Lommele, Laurent Mouchiroud, Matteo Cornaglia Nagi Bioscience SA

The pharmaceutical, chemical, and biomedical industries are undergoing a paradigm shift as ethical, legal, and logistical challenges drive the need to replace traditional vertebrate animal models with innovative New Alternative Methods (NAMs). While NAMs such as organ-on-chip and organoid technologies have revolutionized preclinical research by providing human-relevant organ- and tissue-level insights, there remains a critical need for complementary approaches to address whole-organism phenotypic complexity early in development pipelines.

To address this gap, we present a fully automated, high-data throughput platform leveraging organism-on-chip technology, designed to deliver *in vivo* data at the *in vitro* scale. The platform integrates microfluidics, robotics, and AI-driven analytics to autonomously test up to 64 independent conditions on more than 1,000 *Caenorhabditis elegans* nematodes in parallel. *C. elegans* serves as an ideal 3Rs-compliant model due to its small size, short life cycle, genetic tractability, and high translational relevance to human biology.

The platform fully automates the end-to-end workflow, encompassing organism culture, treatment administration, high-content imaging, real-time data extraction, and phenotypic analysis. Its advanced brightfield and dual-color fluorescence capabilities, combined with AI-based algorithms, enable the precise quantification of diverse phenotypic readouts, including growth dynamics, reproductive health, motility, and lifespan. The system's modular design supports a wide range of applications, including aging research, toxicology, neurodegeneration, and beyond, making it a versatile tool for early-stage safety and efficacy assessment.

By combining the scalability of *in vitro* systems with the biological relevance of *in vivo* models, this platform provides researchers with real-time, actionable insights into complex biological processes. Its ability to deliver high-data throughput, reproducible, and ethically sound data positions it as a powerful complement to existing NAMs, bridging the gap between cell-based assays and vertebrate models.

This technology can empower researchers to streamline discovery pipelines, reduce costs, and enhance predictive accuracy while adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement). By delivering *in vivo* data to the *in vitro* scale, Nagi Bioscience's platform represents a significant advancement in preclinical research, offering a scalable, ethical, and efficient solution for whole-organism phenotypic screening. Its integration of automation, microfluidics, and AI-driven analytics provides a robust framework for accelerating scientific discovery across diverse research fields.

914B A novel anthelmintic compound affects *C. elegans* embryonic morphogenesis

Yasmine Moussa[1], Hala Fahs[2], Fathima Refai[1], Suma Gopinadhan[1], Patricia G. Cipriani[3], Robert D. White[1], Stephan Kremb[1], Xin Xie[1], Yanthe Pearson[1], Antony Page[4], Fabio Piano[3], Kristin C. Gunsalus[31]New York University Abu Dhabi, [2]New York University, [3]New York University, New York University Abu Dhabi, [4]University of Glasgow

Parasitic helminths are a major global health threat, infecting nearly one-fifth of the human population and causing significant losses in livestock and crops. New anthelmintic drugs are needed to combat emerging drug resistance. Using the NYUAD high-throughput screening platform, we screened 50,000 compounds for molecules with broad anthelmintic properties that are non-toxic to a human cell line. The screen identified most known anthelmintics and numerous new compounds, including a new class of avocado-derived fatty alcohols/acetates that we have shown directly inhibits POD-2, a rate-limiting factor in fatty acid biosynthesis (Fahs et al., Nat Comms 2025; doi:10.1038/s41467-024-54965-w). Among the other candidates we discovered D1, a plant-derived pterocarpan compound that caused dose-dependent mortality in C. elegans and P. pacificus across different developmental stages, including embryos. D1 also causes mortality in the multi-drug resistant parasite Haemonchus contortus UGA strain (ruminants). Phenotypic characterization in C. elegans revealed that D1-treated embryos arrest at the two-fold stage. Moreover, embryonic elongation defects were associated with perturbed actin (LifeAct-GFP) and cell junction (DLG-1) organization in embryos. Since other pterocarpans may target kinases, we reasoned that this could be a potential mechanism and hence performed RNA interference screens on genes of interest. We found that lethality was partially rescued through RNAi-mediated silencing of aak-2, encoding AMP-activated protein kinase alpha subunit. Further analysis is ongoing to investigate D1's mode of action during embryogenesis and larval development.

915B Investigating the role of AUP-1 in Proteostasis, Lipid Metabolism and Stress Response

Eirini Mytilinaiou[1], Martina Samiotaki[2], Konstantinos Palikaras[11]Department of Physiology, School of Medicine, National and Kapodistrian University of Athens, [2]Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation Athens

The endoplasmic reticulum (ER) is central to both protein quality control and lipid droplet (LD) regulation, yet the molecular mechanisms linking these processes remain poorly understood. Ancient Ubiquitous Protein 1 (AUP1) is an ER-associated protein that also localizes to the phospholipid monolayer of LDs, suggesting a potential role in coordinating proteostasis and lipid metabolism. Despite evolutionary conservation across multicellular organisms, its precise function has remained elusive. In *Caenorhabditis elegans*, the AUP1 ortholog F44B9.5/AUP-1 is ubiquitously expressed throughout development and aging, exhibiting both diffuse and punctate localization patterns reminiscent of LDs. Loss of *aup-1* results in shortened lifespan and impaired proteostasis, as evidenced by reduced proteasomal function and increased protein aggregation in nematode models of age-related proteinopathies. Moreover, AUP-1 deficient nematodes display heightened sensitivity to heat shock, glucose, and cold stress, potentially linked to lipid composition changes or proteostasis disruptions. Proteomic analysis reveals dysregulation of proteostasis-associated pathways, marked by significant downregulation of ubiquitination and proteasomal degradation components, alongside upregulation of heat shock response and protein folding factors. These findings suggest that AUP-1 plays a multifunctional role at the intersection of proteostasis, lipid metabolism, and stress adaptation. However, further research is needed to elucidate its precise mechanistic contributions to cellular homeostasis.

916B Antioxidant effects of yarrow flower extract in *C. elegans*

Jennifer Newell-Caito[1], Lauren Hume[2], Allison Weymouth[2], Sam Caito[31]Molecular and Biomedical Sciences, University of Maine, [2]University of Maine, [3]Husson University

Yarrow (*Achillea millefolium*) is a small flowering plant found in North America, Europe, and Asia. The dried leaves and flowers of the plant are used medicinally in Indigenous North American culture to make tea as a remedy for cold and flu symptoms. Polyphenols are naturally occurring plant antioxidants that act as a plant's defensive system against oxidation. Flowers in the *Achillea* genus have varying amounts of polyphenols7, but include chlorogenic acid, caffeic acid, rutin, quercetin, luteolin, and apigenin7. Manganese is a known oxidant that causes many neurodegenerative diseases9 including Parkinson's disease. *C. elegans* has been used to examine the toxicity of MnCl2 in Parkinson's due to the conservation of toxicity pathways with mammals. For these reasons, a polyphenolic extract of yarrow flowers (YFE) will be examined for its potential antioxidant properties against oxidative stress induced by MnCl2 in *C. elegans*.

L1 stage worms were pretreated with increasing concentrations of YFE for 30 minutes and washed off. Worms were counted immediately after treatment and again 48 hours later. For survival curve with MnCl2, YFE concentrations were held constant and worms were pre-treated with YFE followed by treatment with varying MnCl2 concentrations for 30 minutes. To assay for antioxidant effects of YFE, DCFDA fluorescence was used to quantify reactive oxygen species (ROS) for 6 hours after worms exposure to MnCl2 in presence or absence of YFE. As oxidative stress is associated aging in C. elegans, lifespan analysis was performed in YFE and MnCl2 treated worms. Following treatments, each population is monitored daily for survival.

Our data show YFE contains polyphenols at a concentration of 1232 +/- 162 mg gallic acid equivalents/mg of dry material. The dose-response survival curve in *C. elegans* was used to determine the LD50, LD20 and, LD10 values to be 1%, 10%, and 58% for YFE, respectively. A survival curve for worms treated with varying concentrations of MnCl2 was shown to have an LD50 value of 37 mM, with a significant increase ($p<0.001$) in worm survival following pre-treatment with 1%, 5%, and 25% YFE. Reactive oxygen species levels were increased in worms treated with 5 mM MnCl2 and 50 mM MnCl2. These values were significantly reduced ($p<0.001$) following pre-treatment with 1%, 5%, and 25% YFE. A *C. elegans* lifespan assay showed 5 mM MnCl2 had a reduced lifespan of 5 days which could be rescued by 10% YFE.

YFE contains phenolic compounds, which are known to act as antioxidants. Pre-treatment of *C. elegans* with YFE reduced levels of ROS that were increased in the presence of MnCl2, protecting *C. elegans* from the effects of oxidative stress. Worms pre-treated with YFE before Mn exposure have an increased lifespan compared to those with only MnCl2 exposure. Overall, this study shows how YFE acts as an antioxidant in MnCl2 induced oxidative stress in *C. elegans.*

917B Pathogenic bacterial attachment to the intestinal epithelia of *C. elegans*

Truc Nguyen, Dalaena Rivera, Robert Luallen Biology, San Diego State University

Wild type Caenorhabditis nematodes can be naturally colonized by a diverse group of bacteria that comprise its gut microbiome. Through environmental sampling, we found a pathogenic bacterium that infects and colonizes the intestine of C. elegans through attachment to epithelial cells. This bacteria was found to be lethal to wild-type C. elegans, causing slow growth, intestinal swelling, intestinal rupture, and ultimately host mortality. We sequenced its genome and identified this bacterium as a new genus and species, which we named Candidatus Lumenectis limosiae (Ca. L. limosiae). We found Ca. L. limosiae can replicate in the gut lumen with anteroposterior directionality in colonization, ultimately resulting in complete colonization of the lumen.

To identify C. elegans genes associated with resistance to Ca. L. limosiae colonization, we conducted a forward genetic screen with the hope to identify adherence factors. We utilized ethyl methanesulfonate (EMS) mutagenesis to induce mutations in WT C. elegans N2 and tested F2s for resistance to infection. As WT C. elegans infected with Ca. L. limosiae take longer than 48 hours to reach L4 stage, we selected mutants based on their phenotypic traits and behaviors at 48 and 72 hours post-infection. Resistance mutants displayed behaviors such as faster motility, darker coloration, and more active feeding activity compared to WT. As a secondary screen, we used fluorescence in situ hybridization (FISH) to test pathogen load. Overall, we successfully isolated two mutants, one that is resistant to colonization by Ca. L. limosiae and another that is likely tolerant to colonization, as it displayed WT behaviors and phenotypic traits while being colonized by Ca. L. limosiae.

Overall, we were interested in finding new genetic factors that are responsible for pathogenic bacterial adherence in the host intestine, but we are likely to hit host immune factors that affect Ca. L. limosiae growth in C.elegans.

918B Quaternary Ammonium Silane Compound K21 Induces Mitophagy, Upregulates Stress Response Pathways, and Extends Lifespan In *C. elegans*

Faye Nourollahi[1], Eunchan Park[2], Jonathan Dietz[2], Mehul Vora[2,3], Christopher Rongo[21]Waksman Institute of Microbiology, Rutgers University, [2]Rutgers University, [3]ModOmics Ltd

Although it is commonly understood that genetically programmed decisions in cell fate dictate the physiology and metabolism of a cell undergoing differentiation, the reverse – how changes in physiology and metabolism can alter cell differentiation – is less well understood. For example, environmentally driven changes in both metabolism and mitochondrial dynamics can influence the differentiation of immune cells in humans. The novel silane derivative K21 can remodel immune cell differentiation to enhance the response to viral, bacterial, and fungal infections, promoting accelerated wound healing possibly by enhancing mitochondrial metabolism and mitophagy. Here, we used genetic, genomic, and cell biological approaches to examine the effects of K21 on metabolic gene expression and mitophagy in *C. elegans*. Mitophagy can be observed in these nematodes using transgenes expressing MitoKeima, a protease-resistant, pH-sensitive fluorescent protein localized to the mitochondrial matrix that is used to monitor mitophagy through dual-excitation fluorescent imaging. We generated a transgene, *odIs167*, that expresses MitoKeima in the *C. elegans* intestine. Intestinal MitoKeima was detectable with 470 nm excitation in the mitochondrial networks of young, well-fed animals. Known inducers of mitophagy created a second population of MitoKeima-labeled spherical structures detectable with 555 nm excitation; these structures could be decorated with Lysotracker green staining. Formation of these acidified mitophagosomes was blocked by mutations in autophagy adaptor *lgg-1*, demonstrating MitoKeima as a faithful in vivo mitophagy reporter. We exposed *odIs167* transgenic animals to a colloidal suspension of K21 layered over an OP50 lawn. We then assessed MitoKeima fluorescence at L4 stage and observed an increase in mitophagy. The K21 suspension did not kill the OP50 food source or impair the ability of nematodes to feed. Nor did treatment impair survival, development, growth, or fertility. Indeed, K21 extended the lifespan of treated nematodes. We used RNA-seq to obtain a transcriptional profile of K21-treated nematodes. GSEA and GO term enrichment analysis showed that K21 induces the expression of genes for lipid beta oxidation, the TCA cycle, OXPHOS/ETC, oxidative stress response, and general mitochondrial and peroxisomal function. Taken together, our results demonstrate that K21 remodels metabolism in *C. elegans* to increase mitophagy, mitochondrial function, and extend lifespan.

919B The effect of Orsay infection and its variants on the fertility of *C. elegans*

Carolynn O'Donnell[1], Solon Aguila[2], Sehrish Khan[1], Abby Reese[1], Jessica Sowa[11]Biology, West Chester University of Pennsylvania, [2]West Chester University of Pennsylvania

Bacterial infections affect the fertility of *C. elegans* by changing the timing of when they have offspring or the overall number of progenies. Viral infections in closely related nematode species were found to alter their reproductive peak. The Orsay Virus is the only known virus to naturally infect *C. elegans*, and our lab has discovered two Orsay Variants, V2 and V13. This project characterizes how V2 and V13 differ from standard Orsay and healthy *C. elegans* in terms of their impact on fertility. This was done by infecting the *C. elegans* with Orsay and its variants and counting the resulting progeny over a ten-day period. We found that there was no significant difference in the average number of offspring per day or when the nematodes stopped reproducing. Orsay however, had a lower overall brood size compared to V2, V13, and mock infection due to a large number of the Orsay infected nematodes dying from their eggs hatching internally, which has not been seen in previous experiments. Through qRT-PCR of the infected nematodes and the viral filtrates we aim to determine whether this effect was due to a difference in viral concentration or a characteristic of the virus itself.

920B A mechanism by which mTORC1 regulates growth, metabolism, and lifespan through mRNA splicing

Takafumi Ogawa[1], T. Keith Blackwell[2,3], Masaki Mizunuma[11]Program of Biotechnology, Graduate School of Integrated Sciences for Life, Hiroshima University, [2]Joslin Diabetes Center, [3]Department of Genetics and Harvard Stem Cell Institute, Harvard Medical School

The evolutionarily conserved serine/threonine kinase complex mTORC1 (mechanistic target of rapamycin complex 1) controls various processes involved in growth and metabolism, and its inactivation extends lifespan across species (Liu and Sabatini, *Nat. Rev. Mol. Cell Biol.,* 2020, Battaglioni *et al., Cell*, 2022). However, due to the complexity of mTORC1 function, its effects on growth and longevity remain to be fully elucidated. Here we employed genetic and bioinformatic screening in *C. elegans* using the orthologues of proteins that had been phosphorylated by mTORC1 in phosphoproteomics studies in yeast and mammalian cells (Huber *et al*., *Genes Dev*. 2009, Robitaille *et al., Science*, 2013, Hsu, *et al., Science*, 2011, Yu, *et al., Science*, 2011, Soulard *et al., Mol. Biol. Cell*, 2010, Schwarz *et al., Mol. Cell Proteomics*, 2015). Through RNA interference screening for mTORC1-associated functions regulating growth and a bioinformatic analysis for the presence of the evolutionarily conserved TOR signaling (TOS) motif (Schalm and Blenis, *Curr. Biol.*, 2002), we identified mRNA splicing-related genes, including RNA-binding serine- and arginine-rich (SR) proteins. We demonstrate that mRNA splicing is activated in response to nutrients through mTORC1, largely independently of its target S6K, suggesting that mTORC1 regulates RNA splicing more broadly than the previously identified S6K-SRPK2 signaling pathway (Lee *et al., Cell*, 2017). mTORC1-mediated regulation of mRNA splicing remodels mRNA structures and gene expression, thereby linking nutrient availability to growth-related processes, including energy and nucleotide metabolism. Moreover, mTORC1 similarly regulates mRNA splicing and expression during growth in human cells. We also demonstrate that although low mTORC1 activity extends lifespan, genetic activation of RNA splicing further extends the lifespan when mTORC1 is attenuated. Thus, maintaining RNA splicing function during aging could provide additional anti-aging benefits.

921B HLH-30/TFEB is necessary for chromatin reorganization and cell cycle arrest upon L1 starvation

Marta Muñoz-Barrera[1], Alejandro Mata-Cabana[1], Nada Al-Refaie[2], Francine A Piubeli[1], Gabriel Gutierrez-Pozo[1], Daphne S Cabianca[2], Maria Olmedo[11]Genetics, University of Sevilla, [2]Institute of Functional Epigenetics, Helmholtz Zentrum München

Cell quiescence is a reversible non-proliferative state maintained by cell cycle inhibitors. This process is crucial for various cell types, particularly for preserving adult stem cell pools, thereby contributing to tissue homeostasis and regeneration. To study cell quiescence in vivo, we use the developmental arrest of *C. elegans* L1 larvae as a model. During late embryogenesis, stem-like cells arrest prior to hatching. When embryos hatch in the absence of food, cells remain arrested until nutrients become available and allow post-embryonic development to commence. Furthermore, adaptation to fasting at the L1 stage also entails a large-scale reorganization of chromatin towards a more repressive conformation.

The transcription factor DAF-16 plays a crucial role during L1 arrest, activating stress response pathways at the beginning of L1 arrest. Its loss severely compromises survival and recovery from L1 starvation and seemed to mediate cell cycle arrest. However, we previously uncoupled the effects of DAF-16 in survival and cell cycle arrest, showing that *daf-16* mutants did arrest cell divisions in complete starvation. This left open the question as to how nutritional information is transduced to arrest cell cycle during L1 starvation. The transcription factor HLH-30 is also involved in metabolic regulation during L1 starvation. Interestingly, depending on the physiological context, DAF-16/FOXO and HLH-30/TFEB function synergistically or have opposing effects. In the case of L1 arrest, their combined effect had never been tested before.

We have performed mRNA-seq analysis of the wild type, *daf-16*, *hlh-30* and *daf-16;hlh-30* mutants at the first day of L1 starvation. Estimation of transcription factor activity from the differentially expressed genes showed that *daf-16* mutants present significant overactivation of HLH-30, while *hlh-30* mutation did not affect DAF-16 activity. Furthermore, the transcription factor HLH-30/TFEB regulates the expression of genes related to cell cycle progression. Indeed, *hlh-30* mutants presented deficient cell cycle arrest in the absence of nutrients, manifested by reduced activation of the cell cycle inhibitor *cki-1*, activation of the S-phase marker PCN-1 and aberrant seam cells division during L1 starvation. We have also observed that HLH-30 is necessary for fasting-induced chromatin reorganization during L1 starvation. These findings unveil novel roles of HLH-30 in cell quiescence and genome architecture in response to starvation.

922B Mammalian GLP-1 receptor agonists decrease lifespan via the dietary restriction pathway in *C. elegans*

Brian Onken, Jack Shannon, Monica Driscoll Rutgers, The State University of New Jersey

Semaglutide, initially used to treat type 2 diabetes (T2D) under the brand name Ozempic (Novo Nordisk), has recently been licensed for the treatment of obesity, resulting in over 9 million prescriptions by U.S. healthcare providers in the last three months of 2022. Semaglutide belongs to a class of drugs known as GLP-1 (glucagon-like peptide-1) receptor agonists that work to improve T2D symptoms by increasing insulin secretion, lowering glucagon secretion, and slowing gastric emptying, thereby improving glycemic control. Importantly, although semaglutide may lower the health risks associated with obesity, little is known about the potential impact of long-term exposure to semaglutide and similar GLP-1 receptor agonists in healthy individuals.

Our previous work demonstrated that reduced caloric intake via dietary restriction (DR) promotes healthy aging in a manner that requires gluconeogenic gene expression, and recent results support that a putative glucagon signaling pathway in *C. elegans* may, like DR, have an overall positive impact on healthspan. As mammalian GLP-1 receptor agonists work to promote insulin signaling and inhibit glucagon pathway activity, we were curious as to whether long-term exposure to a GLP-1 receptor agonist might negatively impact *C. elegans* aging.

Using a commercially available mammalian GLP-1 receptor agonist (GLP-1RA, Sigma), we demonstrated dose-dependent lifespan and healthspan decreases in wild-type *C. elegans* that were exposed to GLP-1RA starting at day 1 of adulthood. In keeping with the weight-loss effects of GLP-1RAs, we found decreased fat stores in treated wild-type animals, although pharyngeal pumping was not noticeably impacted. Lifespan decreases with GLP-1RA are dependent on FOXO class transcription factor DAF-16, suggesting intersection with insulin pathway signaling. Notably, GLP-1RA confers a strong negative impact on animals undergoing DR, with the complete abolition of the long lifespan of *eat-2* DR animals consequent to drug treatment. Supporting that health-promoting gluconeogenic activity is specifically inhibited by GLP-1RA, we found that overexpression of gluconeogenic *pck-2* is sufficient to restore the long lifespan of *eat-2* animals exposed to GLP-1RA. Finally, semaglutide exposure mirrors the detrimental longevity effects of GLP-1RA in both wild-type and DR animals, demonstrating similar lifespan impact of different GLP-1 receptor agonists. Together, these results suggest that GLP-1RA and drugs like semaglutide that function as GLP-1 receptor agonists may have negative health impacts with prolonged exposure through inhibition of DR-related metabolic pathways such as gluconeogenesis. These data are particularly relevant to considerations of the long-term use of GLP-1 receptor agonists to treat obesity or type 2 diabetes, which may involve years- or even decades-long treatment.

923B Nutrient Sensing in Embryonic Sleep

Marvin Onwukwe[1], Pavak Shah[1], Danielle Schmitt[21]University of California Los Angeles, [2]Biochemistry & Chemistry, University of California Los Angeles

In the face of metabolic changes *Caenorhabditis elegans* embryogenesis is resistant to deviating from stereotyped development yet maintains some plasticity to enable adaptation. Embryos broadly express major signaling pathways that sense nutrient availability yet mutants of core nutrient sensors such as AMPK and Akt1 show no gross defects in embryogenesis. Prior work by Evan Ardiel previously demonstrated that *C. elegans* embryos sleep prior to hatching. AMPK and Akt1 are key regulators of sleep in adult worms so we asked whether they also regulate embryonic sleep. To our surprise, AMPK (*aak-1*, tm1944 and *aak-2*, gt33) is dispensable for embryonic sleep, but shows subtle qualitative differences in the pattern of sleep-indicating movement patterns, while Akt1 (*akt-1*, mg144) mutant embryos show an expanded duration of sleep. To support our studies of nutrient-responsive signaling in embryogenesis, we are in the process of integrating a fluorescent reporter of AMPK activity to directly visualize signaling in this pathway.

924C "Incomplete thrashing" - Characterizing a novel behavioral phenotype in a model of adenylosuccinate lyase deficiency

Maia Pappadakis[1], Mia Peifer[1,2], Latisha Franklin[1], Sabrina Sony[1], Wendy Hanna-Rose[11]Pennsylvania State University, [2]University of Pennsylvania

Adenylosuccinate lyase (ADSL) is an enzyme that catalyzes two essential steps in purine biosynthesis, making it important in the production of molecules essential for building nucleic acids and providing energy to a broad range of biological pathways. Deficiency in ADSL function causes a variety of developmental and behavioral phenotypes with variable severity in both humans and *C. elegans*. The pathogenesis of ADSL deficiency has remained elusive, leaving effective treatments for the disease largely unattainable. The enigmatic nature of this pathogenesis can likely be attributed to the multitude of biological functions affected when purine biosynthesis is perturbed. The implications of changes in purine homeostasis—such as the accumulation of intermediates—are not yet fully understood. Using a *C. elegans* model of ADSL deficiency, we aim to uncover the etiology of these broad phenotypes and investigate potential treatments.

Among the various phenotypes observed in ADSL-deficient *C. elegans* is a reduced swimming ability. Not only are affected animals slower, but they also lack coordination while swimming. Wild-type N2 animals thrash in a regular pattern, with the head moving from the ventral plane to the dorsal plane and back rhythmically. Contrarily, ADSL-deficient animals display a much less regular thrashing behavior, occasionally interrupting the rhythmic pattern with the head moving to the halfway point between the dorsal and ventral planes before repeating the direction of its previous head movement. This movement of the head consecutively in the same direction is what we have defined as an "incomplete thrash". Thus far, this previously unreported phenotype has only been observed in ADSL-deficient animals, suggesting an etiology specific to ADSL-deficiency.

Several therapeutic candidates have emerged from screens for drugs that ameliorate ADSL-deficiency phenotypes in yeast. One such drug, disulfiram, has potent efficacy in alleviating some phenotypes in *C. elegans*. The "incomplete thrashing" phenotype previously described is significantly reduced in treated ADSL-deficient animals. Curiously, disulfiram has no effect on other motility phenotypes, such as swimming speed, but does ameliorate some phenotypes believed to be neurologically linked. I will report on behavioral analyses and metabolite quantification to characterize this novel phenotype of incomplete thrashing in ADSL-deficient *C. elegans* and potential connections to broader disease pathogenesis.

925C Adult male sensory neurons produce small and large extracellular vesicles in response to sensory and stress signals

Chanhyun Park, Juan Wang, Maureen Barr Department of Genetics and Human Genetics Institute of NJ, Rutgers University

Extracellular vesicles (EVs) mediate intercellular communication. Cells produce different types of EVs, ranging in size from sub-micron (for example, 100um ectosomes) to cell-sized (for example, 3-4 um exopher) and originating from different biogenic routes. EV cargo content is also diverse, and can promote fitness or pathology. We are using *C. elegans* to understand the fundamental biology of EVs.

A set of 21 male-specific sensory neurons release ectosome-like EVs from sensory cilia (Wang et al., 2014). Using ciliary EV markers, we found that *C. elegans* male-specific neurons also release exophers, large EVs that bud from the cell bodies of CEM neurons in the head and ray RnB neurons in the tail. Exophers play neuroprotective functions in the touch receptor neurons and other tissues (Melentijevic et al., 2017). We are currently examining EV production (ciliary ectosome and exopher) in virgin and mated adult males.

Our preliminary results suggest that L1 starvation increases exopher production in adult males. We will determine how stress (L1 starvation, dauer) affects EV biogenesis of small ciliary ectosomes and large exophers in adult male-specific neurons. The contents of the ciliary ectosomes and exophers will be determined by imaging fluorescently tagged cargo candidates. The long-term goal is to understand the mechanism by which a single cell regulates EV biogenesis (ectosome vs exopher) in healthy and stressful conditions.

926C Exploring the role of microRNA function and proteostasis during aging

Youngyong Park, Amelia F Alessi, Himani Galagali, Margaret R Starostik, John K Kim Biology, Johns Hopkins University

MicroRNAs (miRNAs) are ~21 nucleotide regulatory RNAs that post-transcriptionally modulate gene expression by promoting mRNA degradation or translational repression. In *C. elegans*, the Argonaute proteins ALG-1 and ALG-2 are key mediators of miRNA activity during somatic development. Previous studies suggest that the miRNA pathway plays a crucial role in promoting longevity and maintaining proteostasis. Consistent with these functions, *alg-1(0)* and *alg-2(0)* mutants exhibit shortened lifespan and increased proteotoxicity, including elevated protein aggregation. Notably, aged *alg-2(0)* adults (day 10) accumulate visible protein aggregates, evident from "cloudy" lysates and biochemical fractionation, suggesting a critical role for ALG-2 in preserving proteostasis during aging. To elucidate the underlying mechanisms, we performed mRNA sequencing in day-10 *alg-2(0)* mutants compared to wild-type animals, identifying ~ 900 significantly upregulated transcripts (fold change >1.5, $p<0.05$). Using an aging-related miRNA list (Lencastre et al., *Current Biology*, 2010) and TargetScan analysis, we identified subsets of these transcripts harboring binding sites for miRNAs implicated in lifespan regulation. Among them, the miR-229-66 cluster emerged as a key candidate due to its reported role in low-insulin signaling and dietary restriction-induced longevity via SKN-1/NRF2 (Matai et al., *Aging Cell*, 2023). Of these genes upregulated in *alg-2(0)* mutants, 63—including those encoding F-box proteins, GPCRs, and C-type lectins—contain predicted binding sites for miR-229-66. We are currently validating these targets to assess their contributions to age-associated proteostasis defects. Additionally, our immunoprecipitation/mass spectrometry analyses reveal that ALG-2 potentially interacts with diverse proteins, including RNA-binding proteins and E3 ubiquitin ligases, many of which appear essential for proteostasis based on RNAi assays. These findings support a model in which ALG-2 coordinates aging-associated miRNAs and their targets to regulate proteostasis. Disruptions in Argonaute-miRNA pathway may contribute to protein aggregation and reduced lifespan, with potential implications for age-related neurodegenerative diseases such as Alzheimer's and Parkinson's.

927C Innexin CHE-7 promotes survival and reproductive growth through insulin signaling

Bianca Pereira, Joy Alcedo Department of Biological Sciences, Wayne State University

Innexin CHE-7 forms gap junctions between *C. elegans* neurons [1]. Gap junctions are synapses that allow the flow of electrical signals between cells. Defects in this form of electrical communication can lead to disease. For example, gap junction dysfunctions have been reported in some neurological disorders, like Parkinson's Disease [2]. Thus, understanding the functions of gap junctions should provide insight into how electrical synapses between cells promote health.

In the worm *C. elegans*, we find that innexin CHE-7 ensures survival and reproductive growth. In response to stress, *C. elegans* first-stage larvae (L1s) are induced to switch from reproductive growth to a developmental arrest program known as dauer. Once stress is relieved, dauers exit back into reproductive growth. Here we show that CHE-7 inhibits entry into dauer arrest and modulates the L1 ASJ neuron expression of the insulin-like peptide *ins-6*, which we have previously shown to regulate the switches between growth and dauer arrest [3]. CHE-7 also acts from the ASJ neuron to promote exit from dauer and stimulates the rise in *ins-6* expression in the dauer ASJ neurons prior to exit. Thus, our study uncovers a new layer of regulation of insulin pathway activity, which affects many physiological processes that are important for health.

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928C Life extension via genetic/drug interventions occurs by delaying the onset of aging, not by slowing the rate of aging

Patrick C Phillips[1], Christine Sedore[1], Grace Jackson[1], Gordon Lithgow[2], Monica Driscoll[3]1Institute of Ecology and Evolution, University of Oregon, [2]Buck Institute for Aging Research, [3]Rutgers University

Ever since the discovery more than 30 years ago of mutations capable dramatically increasing lifespan, there has been an increasing push to find genetic pathways and chemical interventions that ameliorate the effects of aging and increase healthspan and longevity. But aging is not merely "getting old," it is an accelerating rate of the decline in function and an increase in the rate of mortality with chronological age. Here, we present a new framework for understanding longevity-extending interventions by dividing the survivorship into two major parts—the onset of aging and the period of accelerated aging—and use a uniform analytical approach to derive these quantities for each of the major mortality models. We apply this new framework to a set of studies with sufficient size to estimate mortality rate, finding that for four genetic and three chemical interventions in *C. elegans* and five genetic and eleven chemical interventions in mice, all treatments that lead to an increase in overall lifespan do so by delaying the onset of aging and none decrease the rate of aging late in life. These effects are readily visualized using a relative hazard and relative lifespan approach. Thus, while there is great interest identify compounds and other approaches to "treat" aging, evidence to date suggests that these interventions do not actually affect aging in the formal sense. These results have important implications for the design and analysis of aging studies, as well as for the field of geroscience as a whole.

929C Microfluidic biocells for investigating *C. elegans* gut-microbiome interactions in spaceflight

Bushra Rahman[1], Atiyya Saroyia[1], Dana Blackburn[2], Audrey Parish[2], Anthony Marino[3], Monica Driscoll[4], Nathaniel Szewczyk[3], Buck Samuel[2], Siva Vanapalli[1]1Chemical Engineering, Texas Tech University, [2]Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, [3]Ohio Musculoskeletal and Neurologic Institute and Department of Biomedical Sciences, Ohio University, [4]Department of Molecular Biology and Biochemistry, Rutgers University

Spaceflight studies in humans and rodents show that microgravity alters the gut microbiome, posing a risk to astronaut health. Understanding how microbiome shifts influence physiology is critical for long-duration space missions. However, these studies face gaps in linking microbiome changes to whole-organism function, as on-orbit experiments are limited by access to live imaging and behavioral assessments. Most prior research has relied on rodent models or cell cultures, which are either resource-intensive or fail to capture integrated physiological function.

As part of the CBIOMES project, which investigates host-microbiome interactions across genetically diverse *C. elegans* strains, we developed NemaCapsules. This is a microfluidic platform configured for spaceflight, enabling simultaneous assessment of *C. elegans* swimming and crawling under identical microbiome conditions.

NemaCapsules provide a fully enclosed, gas-permeable system that enables passive, long-term culturing with minimal astronaut workload. In contrast, traditional microfluidic systems require daily media exchange and crew intervention, making them impractical for spaceflight. By integrating Passive Nematode Culturing (PNC) microfluidics into spaceflight-certified biocell hardware, NemaCapsules form a closed system that supports long-term nematode viability and imaging.

Validation studies demonstrated high survival rates across diverse host strains and microbiomes, confirming the platform's reliability for multi-day spaceflight applications. Locomotory studies further highlight NemaCapsules' ability to examine microbiome-driven effects on physiological health and resilience under microgravity. By enabling high-throughput, on-orbit phenotyping, NemaCapsules advance our ability to investigate how microbiomes modulate host physiology in space—insights vital for astronaut health and understanding adaptation to extreme environments.

930C Deciphering the Survival Strategies of the Non-Model Entomopathogenic Nematode, *Steinernema carpocapsae*, at Rapid Desiccation assisted by Nanoparticle Based Emulsion

Jayashree Ramakrishnan[1,2], Satheeja Santhi Velayudhan[3], Adi Faigenboim[4], Ahmed Nasser[5], Mohammed Samara[5], Eduard Belausov[6], Victoria Reingold[7], Amit Horn[7], Karthik Ananth Mani[8], Guy Mechrez[8], Itamar Glazer[3], David Shapiro ilan[9], Dana Ment[71]Agroecology and Plant health, Hebrew University of jerusalem, [2]Plant Pathology and Weed Research, Agricultural Research Organization, [3]Entomology, Nematology and Chemistry Units, Agricultural Research Organization, [4]Institute of Plant Science, Agricultural Research Organization, [5]Inter-Institutional Analytical Unit, Agricultural Research Organization, [6]department of Ornamental Plants and Agricultural Biotechnology, Agricultural Research Organization, [7]Department of Plant Pathology and weed Research, Agricultural Research Organization, [8]Department of Food science, Agricultural Research Organization, [9]USDA Southeastern Fruit and Tree Nut Research Statiom

Water is essential for survival of all organisms. Terrestrial organisms such as nematodes have evolved specialized adaptations to minimize water loss. Entomopathogenic nematodes (EPNs), such as *Steinernema carpocapsae*, experience rapid desiccation (RD) upon application to foliar surfaces, significantly reducing their biocontrol efficacy. Protective strategies employed by rapidly desiccated EPN and their scope of improvement through formulations has been unexplored. Building on previous results we hypothesized that, Pickering emulsions (Titania Pickering emulsion (TPE) and silica Pickering emulsion gel (SPEG)) enhance EPNs survival and efficacy through distinct fingerprint changes in physiological, and molecular mechanisms. In this study, we established a systematic toolkit to characterize the protective mechanisms employed by *S. carpocapsae* in control and formulated treatments under varying humidity conditions. We determined the effect of RD using gravimetric analysis, confocal microscopy, transcriptomics, ultra-high performance liquid chromatography (UHPLC) and ultrastructural assessments. Our results indicate that, formulated EPNs exhibited significantly delayed water loss and enhanced survival under low humidity compared to controls. Confocal microscopy indicated two distinct protective mechanisms: SPEG primary mode of action is retention of hydration on nematode while, TPE effectively slowed water loss from nematode body. Furthermore, these protective mechanisms correlated strongly with differential patterns of trehalose accumulation, providing biochemical confirmation of formulation efficacy. Transcriptome and ultrastructural analysis highlighted the adaptive mechanisms such as ECM remodeling and cytoskeleton reorganization that encompass as critical components of EPN'S adaptive response to RD.

931C Elucidating the SREBP/Transketolase lipid homeostasis regulatory circuit

Amandine Rapp, Anders Naar, Veerle Rottiers UC Berkeley

The transcriptional activators Sterol Regulatory Element Binding Proteins (SREBP/SBP-1) are master regulators of cellular lipid homeostasis, with key roles in the control of fatty acid and cholesterol production in mammals and *C. elegan*s. Studies in our lab investigating SREBP/SBP-1 function revealed that Transketolase (TKT)-depleted worms exhibit delipidated, *sbp-1(RNAi)*-like phenotypes, revealing a role for TKT in lipid homeostasis in *C. elegans*. The involvement of TKT in lipid homeostasis has also been observed in mice models, where TKT depletion caused reduced fat storage and resistance to diet-induced obesity. TKT is an enzyme catalyzing two reversible reactions in the non-oxidative phase of the pentose phosphate pathway (PPP), bridging this pathway with glycolysis. TKT is thus important for NADPH and ribose production, as well as for energy allocation by allowing the redirection of non-hexose sugars towards glycolysis. Studies in mammalian systems have revealed a non-canonical, transcriptional role of TKT in the nucleus, though whether this happens in *C. elegans* is currently unknown. The mechanism by which TKT depletion causes a reduction in lipid storage has yet to be elucidated. We found that SBP-1 and TKT-1 function are closely related, the expression of *tkt-1* and a wide range of NADPH-producing enzymes being SBP-1 dependent. We also observed that depletion of NADPH-producing enzymes of the PPP (*gspd-1* and 6PGD (T25B9.9)) leads to a strong transcriptional upregulation of enzymes involved in NADPH production, including *tkt-1*. We found that this upregulation is mediated by SBP-1, seemingly through increased nuclear localization. This suggests that SBP-1 responds to decreased NADPH levels, thereby coupling the induction of NADPH-consuming lipogenic processes with those replenishing NADPH pools. Interestingly, TKT-1 was found to contribute to the 6PGD-knockdown-induced upregulation of NADPH production-related genes, including *tkt-1* itself. RNAseq analysis of TKT-1-, SBP-1- and 6PDG-depleted worms showed significant overlap in the transcriptional landscape of *sbp-1* and *tkt-1* worms. The respective contribution of the beforementioned genes in NADPH regeneration is currently being examined through the measurement of whole-body NADP/NADPH ratios in *C. elegans* upon gene knockdown. The hypothesized TKT-lipid homeostasis transcriptional link will soon be investigated using truncated TKT constructs leading to its nuclear or cytoplasmic sequestration.

932C Using *C. elegans* to identify, characterize, and elucidate molecular targets for microsporidia inhibitors

Qingyuan Huang[1,2], Aaron W Reinke[11]University of Toronto, [2]Southwest University

Microsporidia are parasites that infect humans and many agriculturally important animals including bees, shrimp, and fish. Despite the threat these emerging parasites pose to health and food security, few therapeutic options exist. Currently, the only widely used drugs either do not inhibit many species of microsporidia or are associated with host toxicity. Identifying new inhibitors has been challenging due to the lack of a system for rapidly screening compound activity. We have developed a 96-well based liquid assay using *C. elegans* and its natural microsporidian pathogen, *Nematocida parisii*. When *C. elegans* is infected with *N. parisii*, the animal is prevented from making progeny. When an inhibitor is added, this effect is reversed. The inhibitory ability of each compound can be quantified by using a flatbed scanner and automated image analysis to count *C. elegans* progeny. Using this assay, we have screened four compound libraries totaling almost 10,000 compounds, validating over 50 molecules as microsporidia inhibitors. Using a collection of different infection assays, we have determined the mechanisms of these inhibitors as either acting on spores to block invasion or preventing proliferation of the parasite inside of *C. elegans*. By culturing infected worms for multiple generations in the presence of increasing concentrations of an inhibitor, we have evolved drug-resistant *N. parisii* and used whole-genome sequencing to determine inhibitor targets. Together this work shows the usefulness of *C. elegans* in developing a comprehensive framework microsporidia inhibitor discovery and characterization.

933C A conditional raptor mutant that selectively affects bulk protein synthesis to control Hypoxic death

Alison Ritter[1], Cong Xu[1], Chun-Ling Sun[1], Muriel Desbois[2,3], Omar Itani[1], Elyse Christensen[2], Jessica Ho[1], Abraham Correa-Medina[1], Brock Grill[2,4,5], C. Michael Crowder[1,6]1Anesthesiology and Pain Medicine, University of Washington, 2Center for Integrative Brain Research, Seattle Children's Research Institute, 3School of Life Sciences, Keele University, 4Department of Pediatrics, University of Washington, 5Department of Pharmacology, University of Washington, 6Genome Sciences, University of Washington

mTORC1 is a master regulator of anabolic metabolism, responding to nutrients and growth factors to promote cell growth. Inhibitors of mTORC1 are used as immunosuppressants and cancer chemotherapeutics and prolong lifespan of animals ranging from *C. elegans* to rodents. mTORC1 inhibition has been shown to reduce protein synthesis and to activate autophagy. A severe limitation in the study of mTORC1 is the lack of viable loss-of-function alleles of metazoan mTORC1 subunits. In a forward mutagenesis screen for *C. elegans* mutants resistant to hypoxia-induced death, we isolated *gc67,* a viable missense mutation in *daf-15*, which encodes *C. elegans* raptor, a core subunit of mTORC1. Crispr/Cas9 engineering of *daf-15* (*daf-15*(*gc67* Crispr)) as well as *daf-15*(+) transgenic rescue confirmed that *daf-15*(*gc67*) produces hypoxia resistance. *daf-15*(*gc67*) phenotypes are temperature-sensitive: normal development, lifespan and hypoxic sensitivity when cultured at 20°, hypoxia resistant and extended lifespan at 22°, and a fully penetrant larval arrest at 25°. To determine whether induction of autophagy might be responsible for *daf-15*(*gc67*) phenotypes, we estimated autophagy activity using a dual fluorescently-labelled LGG-1 autophagy reporter and observed no increase in autophagosomes in animals cultured at 23°. We also examined nuclear localization of HLH-30, a transcriptional activator of autophagy genes and activated by mTOR RNAi, and found no increase in HLH-30 nuclear localization at 22° but did observe an increase at 25°. To determine whether reduced protein synthesis might be responsible for *daf-15*(*gc67*) phenotypes, we measured incorporation of 35S-methionine and found that the *daf-15*(*gc67*) mutant had significantly reduced protein incorporation at 22° compared to wild type. Likewise, *daf-15*(*gc67*) had a temperature sensitive reduction of puromycin incorporation into nascent proteins and reduced nucleolar-localized FIB-1::GFP expression. Epistasis experiments with the negative translation regulators, *larp-1* and *ncl-1*, found a partial suppression of *daf-15*(*gc67*) hypoxia resistance by *larp-1*(lf) *ncl-1*(lf). On the other hand for larval arrest at 25°, we observed partial suppression by *hlh-30*(lf) but no suppression by *larp-1*(lf) *ncl-1*(lf). We conclude that the *daf-15*(*gc67*) mutation selectively affects protein synthesis to induce hypoxia resistance.

934C Identifying Metabolic Pathways Important for Anoxia Tolerance in Insulin Signaling Mutants: a Multi-Omics Study in *C. elegans*

Jose Robledo, Mary L. Ladage, Rajeev K. Azad, Pamela A. Padilla Biological Sciences, University of North Texas

In humans, oxygen deprivation is central to many health issues that can lead to irreparable complications and diet, or genetics are contributing factors of chronic diseases such as diabetes, cardiovascular, respiratory and metabolic syndromes. Caenorhabditis elegans is oxygen deprivation tolerant and serves as an important model for understanding the molecular mechanisms underlying chronic diseases. We developed a hyperglycemia model for studying oxygen deprivation sensitivity and have found a glucose-supplemented diet can inhibit the oxygen deprivation tolerant insulin like receptor mutant daf-2(e1370). In this study, we aimed to identify necessary compounds that the insulin-like receptor mutant daf-2(e1370) produces for long-term (72 hrs) anoxia survival at the L4 stage and if such metabolites when altered lead to glucose-diet induced sensitivity to oxygen deprivation. Central carbon metabolites were quantified using a targeted metabolomics approach on L4 N2 WT, daf-2(e1370), daf-16(mu86), and daf-2;daf-16 fed either a control or 0.5% glucose-supplemented diet. Metabolomics analysis primarily found trehalose as an abundant metabolite that daf-2(e1370) animals produces, but not our N2 WT, daf-16(mu86), and daf-2;daf-16 animals on any diet. To further explore the regulation of genes in metabolic pathways, RNA Sequencing analysis on L4 IGF-1/insulin-like signaling pathway mutants fed either a control or 0.5% glucose-supplemented diet was performed. Our bioinformatic analysis of daf-2(e1370) animals reveal that the differentially regulated central carbon metabolism genes on both control or 0.5% glucose-supplemented diet alter gene expression differently than when compared to other genotypes. An increase in endogenous trehalose through genetic mutation in the trehalose recycling pathway can enhance long-term anoxia survival; however, not in a glucose-supplemented diet. Simply adding exogenous trehalose supplementation also did not enhance anoxia survival in WT animals. These results further reveal that the daf-2(e1370) enhanced long term anoxia survival phenotype is dependent on functional trehalose metabolism. The results presented further explore the modulation of trehalose metabolism genes impacts the capacity for the daf-2(e1370) glucose-fed animal to survive anoxia. Taken together, our results reveal the importance of trehalose metabolism in anoxia survival.

935C Investigating the effect of Environmental Persistent Free Radicals on the lifespan of *Caenorhabditis elegans*

Victoria H Rodriguez[1], Maha Rehman[2], Fan Zhang[3]1Louisiana State University, 2Biological Science, Louisiana State University, 3Cellular, Developmental, & Integrative Biology, Louisiana State University

Environmentally Persistent Free Radicals (EPFRs) are toxic pollutants generated when aromatic hydrocarbons interact with transition metals during combustion. These pollutants are commonly found in Superfund sites—areas contaminated with hazardous waste that rely on thermal treatment for pollutant remediation. Approximately 23% of the U.S. population lives within three miles of a Superfund site. Previous studies have demonstrated that EPFRs can persist in the environment for extended periods, ranging from weeks to years, and induce oxidative stress by generating reactive oxygen species (ROS), leading to inflammation and tissue damage in mammalian systems. However, their potential effect on aging remains largely unexplored. We hypothesize that EPFR exposure accelerates aging by inducing excessive oxidative stress and cellular damage. To investigate this, we assessed lifespan in *C. elegans* following acute exposure to lab-generated EPFRs on CuO/SiO_2 nanoparticles. Preliminary results indicate that EPFR-containing nanoparticles significantly reduced survival ($p < 0.05$) in a dose-dependent manner, with median survival decreasing by 25% at the highest concentration (10 mg/mL) compared to the nanoparticle-only control. Additionally, EPFR exposure significantly reduced body length in *C. elegans* during early adulthood ($p < 0.001$), whereas the nanoparticle control had no significant effect. These findings suggest that EPFRs negatively impact both lifespan and growth. Future research will focus on characterizing EPFR-induced cellular damage and the affected genetic pathways involved in aging, providing further understanding of how environmental pollutants influence longevity.

936C Environmental and sex-specific regulation of health span in *C. elegans*

Ella B.T. Mayor[1], Veerle Rottiers[21]NST, UC Berkeley, [2]UC Berkeley

Lifespan and health span are both strongly affected by environmental and genetic factors. In addition, most animals, including humans, display sex-specific differences in lifespan and susceptibility to age-related diseases. In *C. elegans*, male lifespan was found to be shortened by male/male interactions, mating and male pheromones while male health span has not been explored at all. Using a single worm liquid assay in 96-well plates, we can measure male lifespan without interactions and without censoring large numbers of males that leave the plates. This assay revealed sex specific differences in the lifespan after germline removal (or in a *glp-1* mutant). However, the liquid assay is somewhat tedious and prone to contamination and papers from the Nishida and Murphy labs have successfully used plate assays to investigate male lifespan traits inspiring us to revisit male lifespan assays on plates. We recently obtained an automated system for monitoring the behavior of group-housed worms throughout their lifespans, called the "C. elegans Observatory", developed at, and provided to us by, Calico Life Sciences LLC. The system measures size and a range of speed measures (e.g. after tapping the plates or when calm) but currently does not analyze lifespan itself automatically. While establishing its use for male health- and lifespan, we can clearly distinguish behavioral traits between males and hermaphrodites. For instance, the calm speed of young males is higher than hermaphrodites but declines quicker. We also find that males health span is more sensitive to environmental conditions such as the addition of FUDR, dead vs live bacteria or the addition of antibiotics. So far, we have tested several established aging paradigms by RNAi depletion such as *daf-2* depletion and moderate mitochondrial inhibition and find that they extend lifespan and speed measures in both sexes. While *glp-1* germline-less males (in contrast to hermaphrodites) do not live longer than controls, recapitulating our liquid assays. We are continuing to test hermaphrodite longevity paradigms not previously tested in males by RNAi and in mutants and will report our progress. In addition, we are working towards an automatic lifespan output using machine learning.

937C Experimental differences within the community confound studies of dauer behavior

Charline Roy[1], Tuan Anh Le[1], Imane Fath Allah[2], Liliane Schoofs[1], Liesbet Temmerman[11]Biology, Division of Animal Physiology and Neurobiology, KU Leuven, [2]Biology, KU Leuven

Behavioral studies in animals are highly dependent on environmental and experimental conditions. *C. elegans* is no exception to this, but while standardized protocols exist to obtain L1–L4 larval stages, dauer induction methods still vary widely across labs (e.g., *daf*-c mutants, starvation, crude/synthetic pheromones), often implicitly assuming all those dauers will lead to comparable results.

Our lab, requiring large populations for peptidomics or semi-automated nictation scoring, induces dauers in small liquid cultures with controlled population density and limited food supply. To establish a reference framework for liquid-cultivated dauers, we assessed how cultivation conditions such as temperature, duration, and parental status influence their physiology and behavior. Notably, young liquid-cultivated dauers (<1 week old) may still be transitioning, appearing dauer-like but failing SDS resistance tests, potentially confounding behavioral results, especially in mutants with delayed dauer entry. Dauer induction in liquid is efficient at 20°C, producing animals actively crawling and performing nictation, a dauer-specific behavior in which nematodes stand on their tail and wave their body. In contrast, 25°C cultivation reduces locomotion and nictation, possibly due to increased heat stress, as indicated by fluorescent reporters.

Moreover, we show that genetic regulation of nictation is cultivation-dependent: *age-1* mutants of the insulin/IGF-1 pathway show increased nictation in 3–5-day-old pheromone-plate-induced dauers but reduced nictation in two-week-old liquid-induced dauers. Similarly, *flp-11* mutants display increased nictation in pheromone-plate-induced dauers but not in liquid-cultivated ones.

Overall, liquid and plate-induced dauers represent distinct paradigms with differing behavioral regulation. For liquid-cultivated dauers, we recommend induction at 20°C, behavioral testing on ≥1-week-old dauers (typically 14 days post induction), and parallel SDS testing to ensure culture quality (>90% resistance in WT). While SDS resistance may exclude mutants with cuticular defects but functional nictation, it prevents misclassification of immature dauers. For the broader dauer community, we highlight relevant factors for discussion to improve reproducibility and comparisons between studies: dauer pheromone exposure strategies, the importance of dauer age, and the risks of selecting dauers based solely on morphology for behavioral studies.

938C Involvement of FMRFamide-like peptides in entry into and recovery from dauer diapause in *C. elegans*

Kanato Sakamoto[1], Yuki Mochida[2], Risako Une[2], Natsumi Kageyama[3], Takashi Iwasaki[2,4], Tsuyoshi Kawano[1,21]The United Graduate School of Agricultural Sciences, Tottori University, Tottori University, [2]Graduate School of Sustainability Science, Tottori University, Tottori University, [3]Tottori University, [4]The United Graduate School of Agriculture, Tottori University, Tottori University

FMRFamide-like peptides (FLPs) are widely conserved in nematodes, and *C. elegans* possesses several FLPs that have been implicated in the regulation of dauer diapause. Dauer diapause is regulated by both insulin-like signaling and TGF-β-like signaling pathways. Our previous research identified several FLP genes associated with entry into dauer diapause through the use of gene-disrupted nematodes. This study demonstrated that FLP-1 and its receptor, FMRFamide-related peptide receptor-1 (FRPR-1), suppress the secretion of DAF-28, the primary ligand of insulin-like signaling, thus promoting dauer diapause.1,2) Conversely, FLP-2 enhances the secretion of INS-35, a potent ligand of insulin-like signaling, which helps prevent dauer diapause.3) Additionally, FLP-3 and FLP-6 also prevent dauer diapause by promoting INS-35 secretion. These FLPs react to environmental growth factors, including dauer pheromones and food. Based on this background, we propose that FLPs may also play a role in recovery from dauer diapause. Given that ASJ neurons are primarily responsible for this recovery process, we will investigate FLPs expressed in these neurons, specifically FLP-15, FLP-21, and FLP-34 (https://cengen.shinyapps.io/CengenApp/).

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939C Investigating the Role of Autophagy in the Barrier Function of Aging Intestines

Maria Sallee, Allison Linkous, Malene Hansen Buck Institute

Autophagy is a critical recycling process that cells use to degrade specific cytosolic materials, including defective proteins and organelles, referred to as cargo. Autophagy generally declines with age, but not in various long-lived mutants; these mutants require autophagy for their extended lifespans, underscoring the importance of autophagy in maintaining organismal health during aging. Intestinal barrier function also declines with age, and our lab previously showed that autophagy is important for maintaining intestinal barrier function during aging (Gelino *et al.,* 2016, PMID: 27414651). However, how autophagy promotes intestinal barrier function is unknown.

To begin addressing this, we first asked how intestinal morphology changes with age in *C. elegans*. Intestinal cells are highly polarized and have specialized apical surfaces facing the lumen, and subapical junction complexes that tightly adhere neighbors together. We observed that aging intestinal cells showed defective localization of key apical and junctional proteins that are important for barrier function, in agreement with previous work (Egge *et al.,* 2019, PMID: 31794717). We additionally found that apical protein mis-localization in individual age-matched animals largely predicted the loss of barrier function. Could autophagy regulate apical and junctional proteins, directly or indirectly, thus contributing to barrier function? To uncover molecular connections between autophagy and barrier function, we are designing a candidate-based RNAi screen to identify specific autophagy proteins that select intestinal cargo important for barrier function.

Interestingly, impaired autophagy and declining intestinal barrier function are also hallmarks of many age-related diseases, such as Alzheimer's and Parkinson's diseases. Some studies have found that poor intestinal health may increase the risk of neurodegenerative diseases later in life (Chen *et al.,* 2016, PMID: 26731277; Skjaerbaek *et al.,* 2021, PMID: 25987282), highlighting the importance of intestinal-neuronal interactions. Therefore, we are also interested in investigating how modulating autophagy and altering intestinal health can impact the health of other tissues and vice versa, in both healthy and diseased aging contexts. These studies will help elucidate the molecular mechanisms that connect autophagy and intestinal barrier function; such insights may help identify new entry points to therapeutically target age-related diseases and improve organismal health.

940C A Functional Atlas of *C. elegans* Steroid Enzymes Links Molting to Repurposed Cholesteryl Ester Metabolism in Nematodes

Amir Sapir, Raj Rani, Or Ben-Hamo, Benjamin Trabelcy, Yoram Gerchman University of Haifa

he metabolism of steroids, such as cholesterol, is critical for mammalian physiology and human health, yet its function in invertebrates remains poorly understood. Using *C. elegans* as a model, we constructed the first comprehensive homology-based enzymatic atlas of steroid metabolism in invertebrates, identifying 159 candidate genes. We conducted a two-dimensional genetic and metabolic screen, knocking down these genes under varying cholesterol levels to identify enzymes specific to steroid metabolism. Among the screen candidates, we focused on *mboa-1*, whose knockdown and knockout disrupted hypodermis and cuticle integrity. *mboa-1* is an ortholog of mammalian SOAT1/2 enzymes, which synthesize cholesteryl esters from sterols and fatty acids. Bacterially expressed *C. elegans* MBOA-1 produced cholesteryl esters when supplemented with the steroid 4,3-cholesta and fatty acids. Moreover, 4,3-cholesta—but not steroid hormones—rescued the *mboa-1* RNAi phenotype, identifying *mboa-1* as part of a novel steroid metabolism branch in invertebrates. Consistent with its loss-of-function phenotypes, *mboa-1* is expressed in seam cells, which contribute to the hypodermis and cuticle, from mid-embryogenesis through larval stages but this expression sharply declines in adults. *mboa-1* expression oscillates with the molting cycle and is regulated by lin-29-mediated heterochronic control during the larval-to-adult transition, underscoring its role in cuticle dynamics. Functional studies in Clade IV and V nematodes, combined with insect expression data, suggest that *mboa-1* has evolved to repurpose cholesteryl ester production from primarily metabolic roles in mammals and insects to structural components essential for nematode cuticle integrity, highlighting a unique aspect of invertebrate steroid metabolism.

941C An AI-Assisted Microfluidic Platform for the Multi-Dimensional Evaluation of Reproductive Toxicity in *C. elegans*

Atiyya Saroyia[1], Taslim Anupom[2], Tyler Chiriboga[2], Siva Vanapalli[31]Texas Tech University, [2]NemaLife Inc, [3]Texas Tech University/NemaLife Inc

Reproductive toxicology (RTox) is essential for assessing pharmaceutical and consumer product safety. *Caenorhabditis elegans* (*C. elegans*) offers an efficient, affordable alternative for RTox studies and has been used to assess chemicals such as heavy metals, herbicides, pesticides, pharmaceuticals, and mycotoxins.

Traditional *C. elegans* culture platforms have limitations that make multi-day reproductive assays tedious. Agar plate assays require daily manual transfers and have poorly defined chemical exposure, while multi-well plates improve uptake but are not well suited for multi-day assays.

Here, we use the previously established microfluidics-based NemaLife platform and apply it for comprehensive evaluation of reproductive toxicity in *C. elegans*. The workflow involves automated high resolution image acquisition of progeny in the microfluidic chip at prescribed time points. The images are uploaded to NemaStudio with a visual AI detecting eggs and larvae to determine progeny counts.

The technology and workflow were validated by exposing *C. elegans* to heavy metal salts (HMS) and comparing lethality and progeny inhibition to well plate studies. Concentration-response curves from 10–12 HMS concentrations over 24 hours showed no significant differences, confirming the NemaLife platform as a reliable RTox alternative.

Next, we investigated whether HMS impacted worms with different reproductive age. L4, day 1, and day 2 adult worms without prior toxin exposure were treated for 24 hours and their brood size and egg-laying rate was determined. Results show that worms with matured reproductive age show significantly greater decline in reproductive function compared to younger ones.

We assessed whether progeny from HMS-exposed maternal worms developed normally. Eggs placed in HMS-free chips showed full viability at 24 hours, but after 72 hours, progeny had significantly reduced body lengths, indicating molecular damage transfer from HMS-treated mothers.

In summary, the NemaLife platform combined with AI-based processing of images, provides a means to deeply phenotype reproductive toxicity due to chemical exposure in *C. elegans*. The platform generates rich data, covering chemical concentrations, reproductive phenotypes, and worm reproductive ages. This comprehensive approach enables early safety and risk assessment of diverse chemicals.

942C Changes in zinc homeostasis influence the innate host defense

Jonathan Saunders, Manish Grover, Franziska Trusch, Michalis Barkoulas Department of Life Sciences, Imperial College London

Zinc is an essential trace element crucial for organismal physiology and numerous biological processes across all domains of life. Its importance in biology stems from its diverse roles as a structural component of proteins, a cofactor for enzymes, and a signalling molecule. Zinc homeostasis is regulated by two families of zinc transporters, the ZIPT and ZnT proteins. These transporters work in opposite directions to maintain optimal zinc concentrations within cellular compartments. Among these, ZIP7 holds a unique position - it is primarily localized to the endoplasmic reticulum (ER) membrane and plays a role in releasing zinc from the ER stores into the cytoplasm. This process can lead to rapid changes in cytoplasmic zinc levels, potentially triggering downstream signalling events. However, how changes in zinc distribution and homeostasis can affect the host innate immune defence is not understood. Here, we investigate this question in the context of oomycete infection, which are fungi-like eukaryotes that naturally infect *C. elegans.* The presence of oomycetes is sensed by *C. elegans* and a transcriptional defence programme, the Oomycete Recognition Response (ORR), is activated.

Through a forward genetic screen, we recovered a mutation in the ZIP7 homologue, *zipt-7.2*, which constitutively activates the ORR and enhances survival upon oomycete infection. We demonstrate that ZIPT-7.2 functions in the epidermis to suppress the immune response. Immune auto-activation relies on OLD-1, an epidermal receptor-tyrosine kinase identified as a key mediator of the ORR, and is associated with increased extracellular zinc levels. A suppressor screen in the *zipt-7.2* mutant background revealed further mutations in the zinc transporters CDF-1 and ZIPT-2.4, which suppress the constitutive activation of the ORR and reduce extracellular zinc levels. Interestingly, we show that pathogen recognition leads to a transient increase in cytosolic zinc levels, which is predicted to antagonise the mounting of the ORR. Our results thus indicate that regulation of zinc homoeostasis has a dual impact on *C. elegans* defence; first, it suppresses basal immunity in the absence of pathogen exposure, and second it may buffer the immune response to prevent excessive induction following pathogen recognition.

943C Natural Variation to Secreted Bacterial Toxins in *C. elegans*

Noah Sciambra, Daniel P Bollen, Read Pukkila-Worley Medicine, UMass Chan Medical School

Infectious pathogens are among the strongest selective forces driving evolution of the human genome. However, the impact of host genetic variation on susceptibility to many pathogens and their virulence factors remains poorly understood. Further investigation on the role of unique genetic mutations in disease susceptibility is critical to advancing our understanding of host-pathogen interactions. *Pseudomonas aeruginosa*, a gram-negative bacterium, is pathogenic to a variety of hosts including humans and *Caenorhabditis elegans*. In humans, *P. aeruginosa* is a leading cause of hospital-acquired pneumonia, and it poses significant risks to immunocompromised people and those with cystic fibrosis. *C. elegans* serves as a powerful model for studying *P. aeruginosa* host-pathogen interactions as it readily consumes and is killed by *P. aeruginosa*. Additionally, the *P. aeruginosa* fast-kill assay, primarily reliant on tricyclic-aromatic toxins called phenazines, serves as an excellent tool for rapid assessment of isolate susceptibility.

We aim to identify evolutionary conserved immune regulators critical for host-pathogen defense by assessing natural variation in susceptibility to bacterial toxins. The *C. elegans* Natural Diversity Resource (CaeNDR) provides a unique platform for conducting Genome-Wide Association Studies (GWAS) in *C. elegans*, offering a database of hundreds of globally-sourced natural isolates with fully sequenced genomes. Here, we present findings from testing CaeNDR Mapping Sets in *P. aeruginosa* fast-kill assays. We report significant natural variation in pathogen susceptibility among the isolates and identify mapped loci harboring potential causative genes. These results highlight the utility of *C. elegans* as a model for uncovering genetic determinants of host-pathogen interactions and provide new insights into the mechanisms of *P. aeruginosa* pathogenicity.

944C Gold sodium thiomalate and 3-bromopyruvate increase lifespan across diverse *Caenorhabditis* genetic backgrounds and reset the transcriptional aging clock toward a "youthful" state

Christine A Sedore[1], Anna Coleman-Hulbert[1], Erik Johnson[1], Erik Segerdell[1], Brian Onken[2], David Hall[3], Yuhua Song[2], Madhuri Achanta[2], Anna Foulger[3], Mustafa Sheikh[3], Theresa FitzGibbon[3], Gordon Lithgow[3], Monica Driscoll[2], Patrick C Phillips[11]University of Oregon, [2]Rutgers University, [3]The Buck Institute

The impetus for the *Caenorhabditis* Intervention Testing Program (CITP) is to identify chemical interventions that robustly and reproducibly extend lifespan and healthspan among genetically diverse species of *Caenorhabditis* nematodes. To this end, we characterized two longevity extending compounds: gold sodium thiomalate (or sodium aurothiomalate, GST) and 3-bromopyruvate (3BP). Gold sodium thiomalate is a candidate longevity-promoting compound predicted by Kapsiana and Howlin (2021) using a computational approach that considered various structural and physiochemical properties as indicators of likely efficacy. 3-bromopyruvate, an anti-cancer agent, decreases glycolytic activity by inhibiting hexokinase, which catalyzes the first step of glycolysis, leading to suppressed ATP production and inhibition of tumor growth in animal models and in at least one human cancer patient. Although long-term toxicity and lifespan/healthspan studies with 3BP treatment have not been reported, 3BP remains a potential dietary restriction mimetic due to its potent inhibition of cellular energy production via glycolysis. Here we show that both GST and 3BP significantly increase lifespan among *Caenorhabditis* strains that are as genetically diverse as the human population. Using bulk RNA-sequencing at a variety of ages, we created a "transcriptional aging clock" using a subset of genes from control individuals that display age-specific patterns of gene expression. Mapping the genes from our treated individuals onto this clock reveals an overall reduction in transcriptional age. These results support the idea that robust longevity-extending interventions can act via global effects across the organism, as revealed at a functional level via changes in gene expression. Using this method, we set the stage for a general approach to define a universal aging clock.

945C The p300/CBP-Associated factor regulates protein homeostasis to promote health and longevity

Avinash Sharma[1], Lina P. Blanco Rodríguez[1], Mai H. To[2], Erica Dinneen[11]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, [2]Department of Biology, Humboldt University of Berlin

Aging is characterized by a gradual decline in both cellular and organismal functions, which is partly driven by epigenetic changes that affect stress responses, proteostasis, and metabolism. We recently demonstrated that a *C. elegans* transgenic strain (tg) that constitutively expresses the p300/CBP-associated factor, PCAF-1—the homolog of mammalian KAT2A/KAT2B—can extend lifespan by 40% in the wild-type background (Silva-García CG, et al. Nature Aging, 2023).

Here, we provide new insights into the mechanisms behind PCAF-1-mediated longevity, highlighting a novel role in maintaining proteostasis and regulating critical stress response pathways. Our transcriptional analyses show alterations in several key stress resistance and longevity-associated genes, including *sod-3*, *hsp-16.2*, *hsp-16.41, hsp-70*, and various glutathione S-transferases (GSTs), along with genes involved in proteostasis and proteasome function. Consistently, worms expressing a polyglutamine tract of length 35 [polyQ(35)] in a *pcaf-1 tg* background exhibit a significant reduction in polyQ aggregates, which improves locomotion and extends lifespan. This suggests that PCAF-1 enhances proteostasis to promote longevity. Furthermore, our epistasis analyses indicate that PCAF-1 genetically interacts with the FOXA transcription factor PHA-4 and the histone deacetylase HDA-1 to influence longevity. While PHA-4 is necessary for facilitating the longevity effects of PCAF-1, HDA-1 works synergistically with PCAF-1. Additionally, we have identified a tissue-specific role of PCAF-1 in promoting longevity in neurons and muscles. Remarkably, our findings suggest that the *pcaf-1 tg* can extend lifespan through different mechanisms, depending on whether the expression is tissue-specific or ubiquitous.

Overall, our study highlights a novel role for this histone acetyltransferase in promoting longevity as a key modulator of proteostasis. It also underscores the importance of exploring different functions of other conserved epigenetic regulators in the context of aging.

946C How environmentally robust is extreme longevity?

Daniel L Shaw, Alyson Fulton, Mirjam Kreutz, Naadia Rashid, Chloe Zenker, Daniel Carolan, Sophia Sachs, Bryce Hochstetler, Sophia Pavlidis, Danielle Berdichevsky, Xiangting Xu, Javier Apfeld Northeastern University

The lifespan of *C. elegans* and many other animals is highly plastic to genetic perturbation. For example, we can increase *C. elegans* lifespan by more than 50% simply by modulating the function of any of 70 different genes. Perhaps most strikingly, null mutations in the *age-1* phosphatidylinositol 3-kinase cause a nearly 10-fold increase in lifespan. How robust is this extreme lifespan extension across different environments?

To address this question, we are growing *age-1* null mutants in different environments. Remarkably, when grown on plates with 2% glucose—an intervention that shortens wild-type lifespan by 20%—the *age-1* null mutants experience an over 80% reduction in lifespan. We have shown that worms grown on high glucose at the beginning of adulthood can recover to live an extremely long life when transferred to standard plates. On the other hand, a shift to plates with high glucose shortens their lifespan dramatically. Interestingly, relative to the time of the shift to glucose plates, the lifespan of the *age-1* mutant is essentially the same, whether the shift was done at the onset of adulthood, one month later, or two months later.

We are currently identifying the extent to which the mechanisms for glucose sensitivity in wildtype animals play a similar role in *age-1* null mutants. We are also extending our analysis to a wider range of compounds and bacterial diets.

947C An FMRF-amide/RF-amide peptide regulates cell non-autonomous protein homeostasis in *C. elegans*

Carrie A Sheeler[1], Jacqueline Lo[2], Daviana Menendez Escalera[3], Emmett Griffin Baldwin[4], Karmen Mah[3], Adam J Roszczyk[5], Antoine E Roux[6], Cynthia E Kenyon[6], Jennifer Garrison[2], Ashley E Frakes[1,71]Genetics and Biochemistry Branch, NIDDK, National Institutes of Health, [2]Buck Institute, [3]NIH, [4]Baylor College of Medicine, [5]University of Rochester College of Medicine, [6]Calico Labs, [7]NINDS, National Institutes of Health

The coordination of protein homeostasis from the brain to peripheral tissues is essential for the health and survival of all organisms. Loss of protein homeostasis is a hallmark of aging and contributes to the development of neurodegenerative disease. In *C. elegans*, glia play a central role in coordinating organismal protein homeostasis and longevity via the unfolded protein response of the endoplasmic reticulum (UPRER). UPRER activation in astrocyte-like glia by *xbp-1s* expression (*glial::xbp-1s*) induces cell non-autonomous activation of the UPRER, which extends lifespan, reprograms peripheral lipid metabolism and renders animals resistant to protein aggregation and chronic ER stress. However, the identity of the signaling molecules required and the full extent of the cell non-autonomous response remained unknown. To determine the organism-wide cell specific transcriptional changes induced by *glial::xbp-1s*, we performed single cell RNA sequencing. We identified tissue-specific upregulation of pathways associated with longevity, development, and metabolism in *glial::xbp-1s* animals compared to controls. Notably, neuropeptide signaling was significantly upregulated in 10 tissue clusters. To identify which neuropeptides drive peripheral UPRER activation, we performed peptidomics on *glial::xbp-1s* and control animals. We identified 25 neuropeptides encoded by 8 genes that were enriched in *glial::xbp-1s* worms. We performed loss- and gain-of-function screens to determine which peptides were necessary and/or sufficient for cell non-autonomous activation of the UPRER. We identified a single neuropeptide, FLP-17, that was sufficient to elicit UPRER activation but was not necessary for UPRER activation in *glial::xbp-1s* animals. While *flp-17* overexpression (*flp-17oe)* did not alter lifespan, we observed increased organismal ER stress resistance and reduced age-associated protein aggregate formation in *flp-17oe* animals. Finally, we determined that FLP-17-dependent UPRER activation relies on signaling through the EGL-6 receptor. In conclusion, we have identified a single neuropeptide, FLP-17, which was sufficient to elicit UPRER activation in peripheral tissues and protect animals from chronic ER stress and protein aggregation. This work demonstrates the importance of neuropeptide signaling in coordinating organismal proteostasis and highlights the complex role glia play in this process.

948C Assessment of Neurotoxic Effects and Involvement in Insulin/IGF-1 Signaling Pathway of *C9orf72*-associated Dipeptide Repeats in *C. elegans*

Min Soo Shin[1], Kazuki Ozawa[1], Yoichi Shinkai[2], Motomichi Doi[21]University of Tsukuba, [2]National Institute of Advanced Industrial Science and Technology

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized not only by motor neuron depletion but also by insulin resistance. While brain insulin resistance is an emerging concept in various neurodegenerative diseases, its underlying mechanisms remain unclear. To elucidate the impact of the Insulin/IGF-1 Signaling (IIS) pathway on ALS, we investigated the toxicity of proline-arginine repeat peptides (*PRs*) produced by the human *C9orf72* gene, one of the primary genetic causes of ALS.

First, we generated a *C. elegans* strain that expresses *PRs* in GABAergic motor neurons. The PR-expressing animals exhibited GABAergic neuronal cell death, disconnected axon morphology, and reduced thrashing behavior, confirming the neurotoxic effects of *PRs* in *C. elegans*. In a *daf-2* mutant background, PR-induced toxicity was alleviated, as evidenced by the restoration of axon morphology and thrashing behavior, suggesting a neuroprotective role of IIS signaling through altered DAF-2 activity against PR toxicity. However, the DAF-2-mediated neuroprotection diminished over time, with significant suppression of thrashing behavior observed between day 1 and day 5 of DAF-2 with *PRs* expression adulthood unlike constitutive DAF-2 mutant. In addition, the constitutive dauer formation observed in *daf-2* mutants remained unaffected by PR expression. However, the PR expression significantly suppressed the longevity phenotype of *daf-2* mutants, despite having no effect on lifespan in the wild-type background. These findings suggest that the intracellular toxicity of *PRs* may accumulate as an aging-related stress specific to IIS pathway.

To investigate the physiological changes underlying the defective phenotypes in PR-expressing animals, we conducted RNA-seq analysis. The GO functional enrichment analysis revealed downregulation of several cellular signaling complex such as proteasomal activity (*rpt-1, rpt-2, rpt-3, rpt-5, rpt-6*) and axon extension (*rpm-1, glo-4*). Notably, the *daf-2* mutation with PR expression alleviated these downregulations, suggesting that IIS activity mitigates PR-induced neurotoxicity in early adulthood. These findings provide transcriptional evidence supporting the neurotoxic effects of *PRs* and the neuroprotective role of IIS on *PRs* expression. The potential interaction between *C9orf72*-related toxicity and altered insulin signaling in neurodegeneration may offer new insights into ALS pathogenesis.

949C Identifying NAD+ Cofactor Imbalances in Response to Stress and Aging

Nicole Sierra, Melanie McReynolds Biochemistry and Molecular Biology, The Pennsylvania State University

Nicotinamide adenine dinucleotide (NAD+) is a vital coenzyme in redox reactions and a key co-substrate for signaling enzymes that drive both bioenergetic and regulatory processes. The transfer of electrons between NAD+ and NADH powers numerous catabolic reactions essential for cellular metabolism. Maintaining a proper balance of these cofactors is critical for metabolic homeostasis, as any disruption can have detrimental effects on cellular function. However, the mechanisms that regulate NAD+ homeostasis remain poorly understood.

Oxidative and reductive stress are major disruptors of redox balance, leading to metabolic dysregulation, particularly with age. The goal of this project is to investigate the key mechanisms that sustain NAD+ metabolism and cellular homeostasis. We hypothesize that disruptions in NAD+ pools within specific subcellular compartments create competition for cofactors, leading to imbalances in redox homeostasis.

To investigate this hypothesis, we conducted brood size and longevity assays in *C. elegans* using the genetically encoded tools LbNOX and mitoLbNOX to induce oxidative stress. Our findings indicate that maintaining a balanced NAD+/NADH ratio is essential for normal reproduction, and redox manipulation leads to significant changes in lifespan. Additionally, we employed high-resolution liquid chromatography-mass spectrometry (HRLC-MS)-based metabolomics to analyze the NAD+ metabolome and track its fluctuations across different developmental stages in LbNOX worms. We predict that shifts in NAD+ compartmentalization will underlie disruptions in homeostasis. This research will provide critical insights into the redox mechanisms that regulate NAD+ metabolism, offering a deeper understanding of how stress responses influence metabolic balance throughout the lifespan.

950C Non-autonomous regulation of age-related morphological changes of the *C. elegans* germline stem cell niche

Nilay Gupta[1,2], Mia Sinks[2], E. Jane Albert Hubbard[21]Department of Biology, New York University, [2]Department of Cell Biology, NYU Grossman School of Medicine

Cellular morphology affects cell function, and aging can alter cell morphology. For cells that participate in contact-dependent cell-cell signaling, such as stem cell niches, age-related changes in cell shape could impair both cell function and tissue homeostasis. To explore how aging affects cell morphology, we are studying the distal tip cell (DTC) in the adult *C. elegans* hermaphrodite. The DTC is the stem cell niche for germline stem cells, and it produces membrane-bound ligands for the germline-expressed Notch receptor, GLP-1, the activity of which specifies germline stem cell fate. The adult hermaphrodite DTC cellular morphology is complex and includes long processes that extend proximally.

We developed a morphometrics pipeline and applied it to live images of the DTC captured over an aging time-course. We found at least three distinct DTC morphology parameters that are radically altered with age: nuclear position, the number and the length of processes. To determine whether/how the insulin/IGF-like signaling (IIS) pathway affects DTC morphology over time, we repeated the aging time-course in *daf-2(rf), daf-16(0),* and *daf-16(0);daf-2(rf)* mutant worms. We found that nuclear displacement occurs independently of *daf-2*, while the reduction in number and length of long processes depends on *daf-2*. In *daf-2(rf)*, DTC length is remarkably stable up to 30 days of adulthood. We also found that DTC process length is not maintained in the *daf-16(0);daf-2(rf)* double mutant, indicating a role for *daf-16* in maintaining DTC process length with age.

Using heterologous expression and auxin-mediated protein degradation approaches, we found that *daf-16* regulates aged DTC morphology non-autonomously, and we identified *myo-3*-expressing muscle, a tissue requirement distinct from that of longevity, as a key contributor. We further uncovered an unanticipated role for muscle-expressed *daf-16(+)* in regulating the germline progenitor (PZ) pool with age, suggesting possible coordinated regulation between the stem cell pool and the niche. However, a simple direct relationship between DTC length and the PZ pool is unlikely since the two do not respond identically to the same *myo-3*-driven *daf-16(+)*. Our results provide general insight into how two components of a stem cell system, the niche and progenitors, that are connected both physically and through signaling, can age differently.

951C XDH-1 inactivation causes xanthine stone formation in *C. elegans* which is inhibited by SULP-4-mediated anion exchange in the excretory cell

Jennifer Snoozy, Kurt Warnhoff, Sushila Bhattacharya, Robin R Fettig, Ashley Van Asma, Chloe Brede Sanford Research

Xanthine dehydrogenase (XDH-1) is a molybdenum cofactor (Moco) requiring enzyme that catabolizes hypoxanthine into xanthine and xanthine into uric acid, the final steps in purine catabolism. Human patients with mutations in *xdh-1* develop xanthinuria which can lead to xanthine stones in the kidney, recurrent urinary tract infections, and renal failure. Currently there are no therapies for treating human XDH-1 deficiency. Thus, understanding mechanisms that maintain purine homeostasis is an important goal of human health. Here, we used the nematode *C. elegans* to model human XDH-1 deficiency using 2 clinically relevant paradigms, Moco deficiency or loss-of-function mutations in *xdh-1.* Both Moco deficiency and *xdh-1* mutations caused the formation of autofluorescent xanthine stones in *C. elegans*. Surprisingly, only 2% of *xdh-1* null mutant *C. elegans* developed a xanthine stone, suggesting additional pathways may regulate this process. To uncover such pathways, we performed a forward genetic screen for mutations that enhance the penetrance of xanthine stone formation in *xdh-1* null mutant *C. elegans*. We isolated multiple loss-of-function mutations in the gene *sulp-4* which encodes a transmembrane transport protein homologous to human SLC26 anion exchange proteins. We demonstrated that SULP-4 acts cell- nonautonomously in the excretory cell to limit xanthine stone accumulation. Interestingly, *sulp-4* mutant phenotypes were suppressed by mutations in genes that encode for cystathionase (*cth-2)* or cysteine dioxygenase (*cdo-1*), members of the sulfur amino acid metabolism pathway required for production of the osmolyte taurine. Furthermore, *cdo-1* mRNA accumulated in *sulp-4* mutant animals, mirroring *cdo-1* activation observed during hyperosmotic stress in *C. elegans* and mammals. We propose that loss of SULP-4-mediated anion exchange causes osmotic stress and *cdo-1* activation, a maladaptive response that promotes xanthine stone accumulation. Supporting the model that the osmotic stress response impacts xanthine stone accumulation, a mutation in *osm-8* that constitutively activates the osmotic stress response, also promoted xanthine stone accumulation in an *xdh-1* mutant background. Thus, our work establishes a *C. elegans* model for human XDH-1 deficiency and identifies *sulp-4* and the osmotic stress response governed by *cdo-1* as critical players in controlling xanthine stone accumulation.

952C Using *Caenorhabditis elegans* to investigate muscle wasting and chemoprotective treatments

Sobha Tumbapo[1], Joshua J Hallesy[1], Sehrish J Khan[1], Emily J Kendall[2], Jana J Stastna[3], Simon C Harvey[4], Marieke J Bloemink[21]Canterbury Christ Church University, [2]Canterbury Christchurch University, [3]Natural and Life Science, Canterbury Christchurch University, [4]University of Greenwich

Sarcopenia, a progressive decrease of skeletal muscle mass and strength, is one of the primary changes associated with ageing and a major cause of physical frailty in the elderly. Rapid loss of muscle mass and strength can also develop during cancer chemotherapy and significantly affect the clinical outcome. Sarcopenia is not well understood at the molecular level, although multiple factors can influence development of sarcopenia, including physical inactivity, and an unbalanced diet.

This study reports the effect of various dietary interventions on the rate of muscle loss using *C. elegans*. We use a transgenic strain (*unc54::gfp*) which has myosin UNC-54 in body wall muscle fluorescently labelled. This allows UNC-54 myosin density to be measured throughout *C. elegans'* lifespan. Average lifespan, motility, and intestinal barrier function (IBF) are also determined during dietary interventions.

The results show mild and medium dietary restriction (DR) increases lifespan and health span, with a delayed onset of muscle wasting and reduced rates of myosin loss compared to control. This indicates sarcopenia is delayed under mild and medium DR due to a reduced rate of myosin UNC-54 degradation, thereby maintaining protein homeostasis.

Supplementation with omega-3 PUFA, has a similar effect, with increased lifespan and delayed onset of sarcopenia. Age-related intestinal barrier function is also improved in omega-3 supplemented worms, with GC-MS confirming uptake of omega-3 PUFA. In the presence of cisplatin, a chemotherapeutic agent, the rate of muscle wasting is accelerated, compared to control, but supplementation with omega-3 PUFA in the presence of cisplatin can partially mitigate this. Overall, these results show dietary intervention can delay onset of age-related diseases such as sarcopenia, by reducing myosin degradation rates, maintaining protein homeostasis and improve intestinal barrier function in *C. elegans*.

953C Ether lipid biosynthesis and aging

Nicole Stuhr[1,2], Alexander Soukas[1,21]Center for Genomic Medicine, Massachusetts General Hospital, [2]Broad Institute of MIT & Harvard

Metformin clinic use started in the 1950s and continues to be the most prescribed drug to treat type 2 diabetes around the world. Metformin is a biguanide that not only lowers blood sugar but also has shown to increase lifespan in preclinical models. Human epidemiological studies have also paralleled these studies, indicating favorable effects of biguanides on promoting lifespan and reducing age-related disease morbidity. Our previous study identified ether lipid biosynthetic pathways as necessary for favorable lifespan-promoting effects from biguanide exposure through the stimulation of ether lipid biosynthesis. Ether lipids require a sophisticated and mechanistically separated biosynthetic pathway that results in a set of distinct molecules with a profound contribution to the structural and functional diversity of bioactive lipids. Loss of the ether lipid biosynthesis machinery mitigates lifespan extension attributed to dietary restriction (through *eat-2*), mTOR inhibition, and mitochondrial electron transport chain inhibition. These data demonstrate how ether lipids form a lynchpin of lifespan regulation and are sufficient to support healthy aging. Building off these findings, we examine the mechanism that metformin and phenformin use to control ether lipid levels in an organism, investigate the timing in which ether lipid synthesis is important for health, in addition to defining species of ether lipids that can promote lifespan and healthspan extension. These results provide an exciting insight on the possibility that modulation of ether lipids pharmacologically or dietarily may provide a novel therapeutic target in aging and age-related diseases.

954C Cell-to-cell transmission of TDP-43 aggregates in *C. elegans* ALS models

Howard Sun, Constantin Bretonneau, Alex Parker Neuroscience, CRCHUM

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease marked by the progressive and selective loss of motor neurons in the nervous system, particularly in the brain and spinal cord. One of several hypotheses suggests that disease-linked proteins, such as TDP-43, can spread throughout the nervous system and lead to the misfolding of other native proteins. Given the potential role of TDP-43 in the pathogenesis of ALS, it is essential to develop tools to examine its propagation within the nervous system. Although emerging data support the TDP-43 propagation hypothesis, studying neuronal transmission in vivo remains challenging. In this context, the C. elegans model organism is proposed for constructing models of TDP-43 Q331K mutants in motor neuron populations to investigate its propagation. The model utilizes a technique called biomolecular fluorescent complementation to observe the protein of interest, TDP-43, in real-time. Two fragmented fluorescent molecules will be linked to TDP-43 variants and expressed in various tissues using promoters to control expression, emitting fluorescence when TDP-43 proteins interact and form aggregates. Preliminary characterization results from the GABAergic neurons to muscle wall cells strain showed a reduced lifespan, an increased paralysis rate, a decreased brood size, and diminished motor capabilities (crawling and swimming). RNA interference trials suggest that inhibiting hsp-90 and hsp-17 slowed the progression of paralysis, whereas inhibition of aak-2 accelerated it. These findings prompted us to begin working with mutants and crossbreeding them to achieve a more consistent and precise phenotype, as observed in the RNAi trials. Additionally, drugs that inhibit the promising pathways identified in earlier experiments will also be tested.

955C Non-canonical roles for autophagy protein ATG-16.2 in neuronal exopher biogenesis and longevity

Ling-Hsuan Sun, Hiroshi Ebata, Malene Hansen Buck Institute for Research on Aging

During macroautophagy (autophagy), cellular components are recycled through the sequestration to autophagosomes (early stage), which merge with acidic lysosomes to ensure degradation (late stage). A key event during the early stage is the conjugation of ATG8 proteins to double membranes via a protein complex that includes ATG16. Recent findings have uncovered novel roles for early-acting autophagy genes involved in conjugation, i.e., in lipidation of alternative vesicles distinct from autophagosomes. Such non-canonical autophagy (NCA) functions may play a role in cellular degradation or secretion, independent of canonical autophagy. Previous studies in mammalian cells have shown that ATG16 can facilitate at least some forms of NCA-related ATG8 conjugation to single membranes, with the C-terminal WD40 domain playing a crucial role. However, the detailed molecular mechanisms of NCA vesicle formation still needs to be fully elucidated. Moreover, while canonical autophagy has been extensively linked to longevity, the potential contribution of NCA functions to aging remains an open and intriguing question.

In our lab's recent research, we discovered that neuronal inhibition of early-acting autophagy genes suppresses polyglutamine (polyQ) aggregation, extends lifespan, and increases exopher biogenesis in *C. elegans* (Yang et al., Nature Aging, 2024). Exopher-genesis is a recently discovered, conserved process that enables the extrusion of especially neurotoxic contents, including polyQ aggregates, directly from the neuronal soma via large membrane-encased vesicles. Notably, these phenotypes rely on ATG-16.2, a *C. elegans* ATG16 ortholog, and its conserved WD40 domain.

To explore whether exopher formation represents a potential NCA event, I am investigating how ATG-16.2 and its WD40 domain regulates this process. In particular, I tested if ATG8 proteins associate with exophers originating from touch neurons, similar to other NCA vesicles. Interestingly, my preliminary data show that *C. elegans* ATG8 proteins localized to exophers, forming luminal puncta-like structures in a lipidation-dependent manner; I am currently analyzing these ATG8-positive structures and how they are regulated by ATG-16.2.

Collectively, my studies suggest that exophers may represent a novel type of NCA vesicles regulated by ATG-16.2. Understanding novel functions for autophagy genes in aging and age-related diseases is important as it may facilitate the development of new effective therapies against these conditions.

956C The Physiological Impact of Cystathionine Beta-Synthase

Karli Paige Sunnergren, Jessica Tanis Biological Sciences, University of Delaware

The amino acid homocysteine (Hcy) is broken down into cystathionine by cystathionine beta-synthase (CBS) or converted to methionine by methionine synthase, which requires vitamin B12 as an essential cofactor. Low vitamin B12 and CBS deficiency can cause hyperhomocysteinemia (HHcy), which is characterized by an abnormally high level of total Hcy in the blood. Additionally, CBS is one of three enzymes responsible for producing the gaseous signaling molecule hydrogen sulfide (H2S), and the major producer of H2S in the brain. HHcy and dysregulated H2S are risk factors for a multitude of age-related diseases including cardiovascular disease, vascular dementia, and Alzheimer's disease (AD), although the mechanisms underlying the connections between increased Hcy, dysregulated H2S, and these pathologies are not well understood. We have knocked out *cbs-1* in *C. elegans*, which is 54% identical to human CBS. Loss of *cbs-1* results in increased Hcy, which is reduced with B12 supplementation due to increased conversion of Hcy to methionine, and decreased levels of H2S regardless of B12 status. *cbs-1* mutants exhibit delayed development, reduced brood size, decreased body length, and extended lifespan, all of which return to wild-type levels when the animals are supplemented with B12. We have also discovered that *cbs-1* mutants are resistant to paralysis when exposed to the acetylcholinesterase inhibitor aldicarb, suggesting decreased acetylcholine (ACh) signaling at the neuromuscular junction. The aldicarb resistance phenotype is not impacted by vitamin B12 status, suggesting a role for H2S in regulating cholinergic signaling. Imaging of *cbs-1* mutants expressing RFP to label the cholinergic motor neurons revealed morphological defects in the dendrites of these neurons. Together, these results suggest that decreased levels of H2S, resulting from loss of CBS, may decrease ACh release from cholinergic neurons, providing new insight into a potential mechanism by which CBS-mediated H2S signaling impacts age-related cholinergic signaling decline and risk for AD.

957C Simple in-cell processing enables deep proteome analysis of a low-number of *C. elegans*

Malek Elsayyid[1], Yanbao Yu[2], Jessica Tanis[11]Biological Sciences, University of Delaware, [2]Chemistry and Biochemistry, University of Delaware

To examine the *C. elegans* proteome, worms are typically lysed to extract proteins, then subjected to proteolytic digestion followed by liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis. The worm cuticle complicates extraction of intracellular proteins, such that the animals are usually flash frozen in harsh lysis buffer followed by grinding or sonication to obtain sufficient protein yield. However, such physical disruption can cause significant loss of material, which increases the number of worms required, and introduces technical variation in sample preparation that can impact downstream data interpretation. Here we present an on-filter in-cell (OFIC) processing approach, which can digest *C. elegans* proteins directly in the cells of the organism after methanol fixation. With OFIC processing and single-shot LCMS analysis, we identified over 9,400 proteins from a sample of only 200 worms, the largest *C. elegans* proteome reported to date that did not require fractionation or enrichment. We systematically evaluated the performance of the OFIC approach by comparing it with conventional lysis-based methods. Our data suggest superior performance of OFIC processing for *C. elegans* proteome identification and quantitation, with no bias toward any subcellular protein groups or tissues. We further evaluated the OFIC approach with even lower input samples and identified on average 2,500 proteins from a single worm, nearly 4,400 proteins from three worms, and over 6,000 proteins from five worms. We next determined the feasibility of using OFIC digestion on *C. elegans* embryos, which are physically protected by an eggshell and contain a large amount of maternal yolk proteins, presenting significant hurdles for low-abundance protein identification, and identified nearly 7,500 proteins with single-shot LCMS. Finally, we used this method to determine how the proteome is impacted by loss of superoxide dismutase (*sod-1)*, the ortholog of human *SOD1*, a gene associated with amyotrophic lateral sclerosis (ALS). Analysis of 8,800 proteins from only 50 worms as the initial input showed that loss of *sod-1* affects the abundance of proteins required for stress response, ribosome biogenesis, and metabolism. In conclusion, our streamlined OFIC approach minimizes sample loss while offering the simplest workflow reported to date for *C. elegans* proteomics.

958C Lactate promotes longevity via acetylation and redox-dependent metabolic changes

Arnaud Tauffenberger[1], Payton Netherland[1], Hubert Fiumelli[2], Frank Schroeder[1], Pierre Magistretti[31]Boyce Thompson Institute, [2]Bioscience, King Abdullah University of Science and Technology, [3]King Abdullah University of Science and Technology

Aging is a malleable process that can be modulated by genetic, dietary, and pharmacological interventions. A hallmark of aging is the decline in energy metabolism efficiency, particularly in maintaining intracellular homeostasis. L-lactate has emerged as a multifaceted molecule, influencing diverse cellular functions from reducing inflammation to enhancing muscle biogenesis and synaptic plasticity. Notably, L-lactate has been shown to improve stress resilience and extend longevity in *C. elegans*, yet the molecular mechanisms underlying these effects remain elusive. In this comprehensive study, we employed untargeted metabolomics and transcriptomics to decipher the lactate-induced changes in the transcriptional network governing aging. By profiling nematodes at multiple time points during adulthood, we uncovered that lactate supplementation significantly modulates cellular redox state and protein acetylation—both critical for longevity extension. Our genetic screening identified the NAD-dependent deacetylase SIR-2.1 as a key regulator of lactate-mediated longevity, providing a novel mechanistic insight. Our multi-omics approach revealed a biphasic response to lactate: early metabolic alterations primarily affecting lipid metabolism, followed by mid-life transcriptional changes impacting signaling cascades associated with cellular homeostasis (proteasome function, redox balance, and detoxification mechanisms). Intriguingly, we observed a marked reduction in metabolic processes, including mitochondrial respiration and lipid metabolism, suggesting a potential metabolic reprogramming event. This integrative analysis has significantly advanced our understanding of how lactate influences cellular physiology and homeostasis. Our findings not only elucidate the mechanisms of lactate-induced longevity in *C. elegans* but also unveil potential targets for validation in higher organisms. These insights may have far-reaching implications for cell survival strategies, particularly in the context of neurological disorders, opening new avenues for therapeutic interventions in age-related diseases.

959C Modular small molecules play a central role in *C. elegans* physiology

Arnaud Tauffenberger, Payton Netherland, Chester Wrobel, Frank Schroeder Boyce Thompson Institute

Cellular metabolism is a complex, intricate network of enzymes and molecules influencing all aspects of cellular homeostasis. The scientific community has just begun to understand that decades of intense research have only revealed 10% of the entire metabolome in most species. Recent studies indicate that the metabolome of *C. elegans* and other animal model systems may include >100,000 compounds, representing a largely untapped reservoir of chemical diversity and bioactivities. A prime example of the physiological impact of small molecules, the ascarosides (an ascarylose sugar linked to a hydroxylated short-chain fatty acid) regulate aging, development, and behavior. More recent work has revealed previously uncharacterized metabolites derived from primary metabolism and catabolic pathways, including amino acid and nucleoside degradation products. Using untargeted metabolomics, several teams have identified a family of carboxylesterases (CEST) responsible for the biosynthesis of a large class of these novel metabolites called *mo*dular *gl*ucosides (MOGLs). This large and diverse family of small molecules is based on a glucoside scaffold decorated with diverse moieties from endogenous metabolism and bacteria-derived compounds, like indole, which is toxic at high concentrations.

However, the role of MOGLs in cellular homeostasis remained unclear. Our recent work investigated how these small molecules influence stress resistance and longevity. We screened mutants of CEST enzymes responsible for MOGL biosynthesis using a combination of phenotyping, transcriptomics, and metabolomics. First, we observed that the abundance of a subset of MOGLs increases with age, while CEST loss of function dramatically impacts stress resistance and longevity in various contexts. We further observed that exposure to different stresses (oxidative, heat, starvation) increased MOGLs abundance, in a stress-specific manner. Understanding the role of these small molecules as signaling molecules or part of detoxification mechanisms will help decipher their role in cellular physiology and may open new ways to identify biomarkers of stress, aging, or age-related dysfunction.

960C Cold shock induces the formation of stress granules via HSF-1

Piyathida Tawornparcha, Tsui-Ting Ching, Ao-Lin Hsu Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University

Stress granules (SG) are cytosolic, membrane-less condensates composed of RNA and proteins. They form in response to stress, resulting in a pause in the translation process to conserve energy for other survival pathways. Stressors that can induce SG formation include heat shock, starvation, dietary restriction (DR), hypoxia, viral infections, toxins, and other factors that affect protein translation. We have previously reported the pathways required by different types of stressors to induce SG formation. Specifically, HSF-1 (heat shock factor 1) is required for heat shock- and starvation-induced SG formation, while the AMPK-eEF2K pathway is necessary for starvation- and DR-induced SG formation. However, a recent report has identified a new type of stress that can induce SG formation in yeast and mammalian cells: cold shock. Since cold shock is the direct opposite of heat shock in terms of temperature change, we wondered whether their dynamics and key regulators are the same. We found that both heat shock and cold shock are fast inducers of SG formation in *Caenorhabditis elegans*. However, cold shock requires a longer period to reach maximum induction compared to heat shock (30 minutes at 4°C vs. 15 minutes at 37°C). Despite these differences in dynamics, both heat shock and cold shock induce SG formation via HSF-1. Knocking down HSF-1 completely abolished SG formation.

961C Uncovering the role of ECPS-1/Ecm29 in proteasome regulation.

Sarah Tomlin[1,2], Irini Topalidou[3], Nicolas J Lehrbach[2,31]Fred Hutchinson Cancer Research Center, [2]University of Washington, [3]Basic Sciences, Fred Hutch Cancer Center

Proteostasis is essential for proper cellular function. The ubiquitin-proteasome system (UPS) plays a central role in proteostasis by degrading damaged, misfolded or excess proteins. Dysregulation of proteasomal degradation contributes to the pathogenesis of diseases including neurodegeneration and cancer. Despite the importance of proteasome regulation in proteostasis, the mechanisms ensuring adequate proteasome activity across cellular and developmental contexts and remain poorly understood. My work utilizes the powerful genetics of *C. elegans* to elucidate how ECPS-1/Ecm29/ECPAS, a conserved proteasome-binding protein, regulates proteasome function. I will present results revealing that ECPS-1 is required to ensure adequate proteasome function *in vivo*. These findings contrast with previous yeast and cell culture studies suggesting that binding of Ecm29/ECPAS to proteasomes inhibits their function. ECPS-1/Ecm29 is a large scaffolding protein that lacks any domains of known function; the so mechanisms by which ECPS-1 binding could lead to distinct effects on proteasome function are unclear. To understand how ECPS-1 binds to and regulates proteasomes, I have generated animals expressing mutant forms of ECPS-1 harboring truncation or internal deletions, which I am analyzing for their ability to (1) bind to proteasomes and (2) either inhibit or promote proteasome function *in vivo*. Ultimately, this research aims to uncover fundamental mechanisms governing proteostasis and explore their implications for healthy aging and potentially inform future therapies for neurodegenerative diseases.

962C Identifying genes that mediate the lifespan shortening effect induced by mix-culturing

Yu Ting Tu[1], Tzu-Ying Liu[2], Tsui-Ting Ching[2], Ao-Lin Hsu[11]Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

Aging is a complex process defined by progressive decline of various physiological functions. In addition to being regulated by intrinsic genetic factors, the aging process is also influenced by external environmental factors. Our previous studies have shown that when hermaphroditic *C. elegans* sense male pheromones, their lifespan is shortened. Inspired by this, we set out to test the effects of co-culturing long-lived mutants with wild-type worms on their lifespan. Intriguingly, we found that the lifespan of DR-mimicking *eat-2* mutants is significantly shortened when co-culturing with the wild-type animals. We also found that his effect might be mediated by pheromones released by the wild-type worms. To further understand the molecular underlying mechanism that may mediate this effect, we performed RNA sequencing to identify gene expression changes resulting from the mixed cultivation of these two strains. We designed a new analytical method to quantify the deviation of gene expression from the expected average after co-culturing, allowing us to evaluate the effect of mix-culturing on each gene with regards to their mRNA expression. Using the method, we have identified genes whose expression shifted toward either WT animals or *eat-2* mutants when co-culturing together.

963C Diet-Driven Adaptations in *C. elegans*: Impact of the bacterial food sources and fatty acids on physiology and behavior

Serhat Turkmen, Ly T. T. Luu, Adrian Garibay, HaoSheng Sun Cell, Developmental and Integrative Biology, University of Alabama at Birmingham

Diet is a fundamental determinant of metabolism, development, and behavior, shaping physiological processes across generations through complex nutritional and molecular interactions. Different *Escherichia coli* dietary strains (OP50, NA22, and HB101) vary in nutritional composition, influencing *Caenorhabditis elegans (C. elegans)* reproduction, lifespan, and behavior. Polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA, 20:5*n-3*), play a crucial role in modulating these physiological traits. The *fat-4* mutant strain, which lacks Δ5-desaturase and is unable to synthesize arachidonic acid (ARA, 20:4*n-6*) and EPA, provides a valuable model to dissect the specific contributions of these long-chain PUFAs to metabolic and behavioral plasticity in response to dietary variation. Here, we leverage the advantages of *C. elegans* to investigate the long-term dietary effects on metabolism and development.

To investigate dietary effects, we maintained wild-type *C. elegans* on OP50, NA22, and HB101 for over 20 generations and assessed reproduction, growth, lifespan, and behavior across different developmental stages. To evaluate phenotypic plasticity, we introduced a dietary switch to OP50 at L1 or L4 stages and measured the resulting changes. Additionally, to assess how endogenous fatty acid synthesis interacts with dietary lipid composition, we conducted parallel experiments using *fat-4* mutants. We compared fatty acid profiles, reproductive output, developmental timing, and morphological and locomotion differences in *fat-4* mutants vs. controls. We also performed EPA supplementation at various developmental stages to assess its ability to rescue phenotypic defects in *fat-4* mutants. Our results demonstrate that bacterial strain selection and PUFAs have profound and lasting effects on *C. elegans*, with implications for metabolic programming. Our ultimate goal is to unravel the interplay between dietary nutrients, lipid metabolism, and physiological adaptation in *C. elegans*.

964C The FMRFamide-like peptide-11 (FLP-11) plays a crucial role in promoting larval diapause in *Caenorhabditis elegans* by suppressing the production and secretion of DAF-7, a TGF-β-like molecule

Riko Uegaki, Takashi Iwasaki, Tsuyoshi Kawano The United Graduate School of Agricultural Sciences, Tottori University, Tottori University

FMRFamide-like peptides (FLPs) are widely conserved in nematodes. *C. elegans* also possesses FLPs, some of which a have been reported to be involved in dauer diapause. Dauer diapause is regulated by both insulin-like signaling and TGF-β-like signaling pathways. Previous studies have shown that FLP-1 suppresses the secretion of DAF-28, the primary ligand of insulin-like signaling, thereby promoting dauer diapause.1) In contrast, FLP-2 promotes the secretion of INS-35, a potent ligand of insulin-like signaling, which helps prevent dauer diapause.2)

In this study, we demonstrate the involvement of FLP-11 in dauer diapause through its regulation of DAF-7, the sole ligand of the TGF-β-like signaling pathway relevant to larval development. Disruption of the flp-11 gene prevented dauer diapause, while overexpression of flp-11 promoted it. Epistasis analyses indicated that flp-11 functions specifically within the TGF-β-like signaling pathway, rather than the insulin-like signaling pathway. FLP-11 suppresses the production and secretion of DAF-7, thereby promoting dauer diapause. Additionally, the production and secretion of FLP-11 were found to increase in response to dauer pheromone and decrease in the presence of food. Future research will focus on identifying the receptor for FLP-11.

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965C Conserved Role of MCA-1 and PMCA in Orchestrating Precontractile Calcium Elevation: Insights from *C. elegans* to Human Myocytes

Andres G. Vidal-Gadea[1], Damiano Gabriel Marchiafava[1], Wolfgang Vidal Stein[1], Martin Engelke[21]School of Biological Sciences, Illinois State University, [2]Biochemistry & Cellular and Molecular Biology, The University of Tennessee-Knoxville

Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder marked by progressive muscle degeneration and aberrant calcium homeostasis. Here, we employ a synergistic cross-species approach using Caenorhabditis elegans and human myocyte cultures to elucidate mechanisms underlying early calcium dysregulation in dystrophic muscle. In C. elegans, dystrophic embryos exhibit premature and elevated sarcoplasmic calcium levels prior to muscle contraction, accompanied by delayed hatching and early muscle weakness. RNA interference-mediated knockdown of the transmembrane calcium efflux pump MCA-1 selectively increased calcium in wildtype worms, implicating impaired MCA-1 function in the dystrophic phenotype. Complementary studies in human myocytes revealed that dystrophic myotubes not only display delayed differentiation but also sustain abnormally high intracellular calcium levels relative to healthy controls. Critically, dystrophic human myoblasts showed reduced expression of Plasma Membrane Calcium ATPases (PMCA1 and PMCA4), and targeted silencing of these pumps in healthy cells mimicked the calcium elevation observed in dystrophic cultures. Together, these findings establish a conserved role for MCA-1 in C. elegans and PMCA1/4 in human muscle in regulating early calcium dynamics, providing new insights into the molecular underpinnings of DMD. By identifying these calcium regulators as potential contributors to the initiation of dystrophic pathology, our study highlights promising molecular targets for therapeutic intervention aimed at ameliorating calcium-induced muscle degeneration in DMD

966C Natural Microbes Induced Internal Egg Hatching in *Caenorhabditis elegans*

Nora Villafuerte, Fan Zhang Cellular, Developmental, & Integrative Biology, Louisiana State University

In natural environments, *Caenorhabditis elegans* interacts with a diverse microbial community, making it an ideal model for studying the genetic basis of microbiome-host interactions. In this study, we investigated the effects of bacterial strains from the *C. elegans* natural microbiota (CeMbio) on host reproduction and lifespan. We identified four bacterial strains: *Brucella pituitosa* (BH3), *Lelliottia amnigena* (JUb66), *Pantoea nemavictus* (BIGb0393), and *Enterobacter hormaechei* (CEent1) that significantly increase in internal egg hatching during late reproductive adulthood, affecting 30% to 70% of the population, compared to less than 10% when grown on *Escherichia coli* (OP50). Internal egg hatching has previously been linked with nutrient deprivation and bacterial pathogenesis, with insulin signaling playing a key role in both conditions, prompting us to explore the role of insulin signaling in this process. Using daf-2/IGFR loss-of-function mutants, we observed increased internal egg hatching in the Class 2 mutation *daf-2*(e1370) allele, which affects tyrosine kinase binding domain, when exposed to BH3, BIGb0393, and OP50 compared to N2. In contrast, the Class 1 mutation *daf-2*(e1368) allele, which disrupts extracellular ligand binding domain, exhibited reduced internal egg hatching on BH3, JUb66, and CEent1, with no change on OP50. These findings suggest that bacterial modulation is acting on extracellular ligand binding domain and dependent on downstream transcriptional factor DAF-16/FOXO. We observed no difference in internal egg hatching when the DAF-16/FOXO loss-of-function mutant *daf-16*(mgDf50) allele was exposed to these bacteria strains compared to N2. Interestingly, there was a decrease in internal egg hatching when the *daf-16*(mgDf50)*daf-2*(e1370) double mutant allele was exposed to the four natural microbes, but not to OP50. We further found that N2 exhibited a reduced lifespan on all tested natural microbial strains except for BH3, which supported a lifespan comparable to OP50. This suggests that microbial species not only influence reproduction but also plays a key role in host longevity. Our findings highlight the role of natural bacterial communities in modulating host reproduction and lifespan through insulin signaling in *C. elegans*, with implications for microbial roles in the host evolutionary transition from oviparity to vivipary.

967C The IRE-1-XBP-1 and PEK-1 signaling pathways mediate protection against the mitis group streptococci in *C. elegans*

Alissa Villhauer[1], Boopathi Balasubramaniam[1], Julia Stefanik[1], Ransome van der Hoeven[1,2]1Iowa Institute for Oral Health Research, University of Iowa College of Dentistry and Dental Clinics, [2]Periodontics, University of Iowa College of Dentistry and Dental Clinics

Members of the mitis group streptococci are inhabitants of the oropharyngeal cavity and have been shown to be causative agents of a variety of human diseases, such as infective endocarditis, community-acquired pneumonia and, more recently, bacteremia in patients with neutropenia. The mitis group is known to produce hydrogen peroxide (H2O2) as a virulence factor. A few studies have highlighted a role for H2O2 as a cytotoxin that induces macrophage and epithelial cell death. More importantly, we have shown that this group of opportunistic pathogens mediates killing of *Caenorhabditis elegans* via H2O2. Furthermore, we have shown the endoplasmic reticulum (ER) transmembrane protein kinase IRE-1 is required for the survival of the worms on *S. gordonii,* a representative of the mitis group, suggesting the ER unfolded protein response (UPRER) plays an important role during infection. In this study we investigated whether the three branches of the UPRER are activated in response to streptococcal infections. To determine the activation of the UPRER chaperone BiP (Hsp-4) in the worms, we compared the expression of *hsp-4* fused to green fluorescent protein in the presence of *S. gordonii* wild-type (WT), *S. gordonii ΔspxB*, and *E. coli* OP50. In addition, we compared the survival of *hsp-4* knockdown worms relative to the vector control treatment on *S. gordonii* WT. To determine if *xbp-1, atf-6* and *pek-1* were required for the survival of the worms on *S. gordonii* WT, we compared the survival of an *xbp-1* mutant to the wild-type worms. To determine the activation of *pek-1*, we compared the phosphorylation of eIF-2α in the presence of *S. gordonii* WT and ΔspxB. A significant expression of *hsp-4*::GFP was observed in worms exposed to *S. gordonii* WT relative to the Δ*spxB*, and *E. coli* OP50. Knockdown of *hsp-4* resulted in a significant decrease in the survival of the worms compared to the vector control treated worms. A significant decrease in the survival of the worms was observed for the *pek-1* and *xbp-1* mutant worms relative to the wild-type worms. Phosphorylation of eIF-2α was significantly increased in the worms exposed to the *S. gordonii* WT relative to the Δ*spxB* mutant strain. Taken together the data suggests the IRE-1-XBP-1 and PEK-1 regulated branches of the UPRER mediate protection of the worms against *S. gordonii* WT.

968C Formation and Dynamics of Biomolecular Condensates in *C. elegans*

Levi von Kalbe University of Hamburg

Biomolecular condensates are membraneless intracellular assemblies that are formed via liquid-liquid phase separation (LLPS). Biopolymers like RNA or proteins aggregate in stress and age-related condensates called stress granules (SGs) that have important functions like safeguarding the cell from damage and maintaining the homeostasis necessary for biochemical reactions. In *C. elegans*, the genetic and structural dynamics of biomolecular condensates like SGs and P granules have been widely investigated. Because of their size, imaging of SGs requires super-resolution microscopy and fixation of the animals. As chemical fixation often leads to tissue damage, structural analysis in the native tissue state has been challenging. BIKON is an interdisciplinary research consortium studying the formation and dynamics of molecular condensates in plants and animals. In our part of BIKON, we aim to establish a new workflow that combines the analysis of condensate dynamics by super-resolution cryo-fluorescent microscopy (SR-cryo-FM) and cryo-electron tomography (cryo-ET) with high-pressure freezing of the worms in vitreous ice to achieve a near-natural state. Fluorescent constructs of genes related to stress or aging will be utilized in focused ion beam scanning electron microscopy (FIB-SEM) to correlate the microscopy data, find regions with accumulations of SGs, and analyze them in their subcellular context. Mutant strains with abnormalities in stress regulation and aging can be compared to wild-type animals to dissect the dynamics of SGs in different conditions. Furthermore, the interchange of molecules like RNA and proteins between different molecular condensates, such as SGs and the constitutively formed P-bodies, will be examined. As biomolecular condensates have been linked to many developmental processes and a variety of diseases, their analysis under normal and pathological conditions can elucidate our understanding and find potential treatments.

969C SAM-synthase specific effects on metabolism and mitophagy underlie resistance to heat stress in *C. elegans*

Athena Munden[1], Dominuque Lui[1], Dana Miller[2], Amy K Walker[31]PMM, UMASS Chan, [2]Biochemistry, University of Washington, [3]Program in Molecular Medicine, UMASS Medical School

Methylation is a central regulatory mechanism that bridges methionine and folate metabolism to gene expression via histone modifications and to membrane dynamics through phosphatidylcholine (PC) synthesis. In mammals, SAM is produced by two synthases—liver-specific MAT1A and the essential MAT2A—whereas in *C. elegans* this enzyme family has expanded to four members (*sams-1, sams-3, sams-4*, and *sams*-5), providing a unique opportunity to dissect pathway-specific functions. Notably, while knockdown of each synthase reduces SAM levels to a similar degree, loss of *sams-1* uniquely extends lifespan and enhances heat stress survival, in contrast to the rapid lethality observed with stressed *sams-4*deficiency. An integrated multi-omics approach—encompassing transcriptomics, epigenomics, lipidomics, metabolomics—reveals that *sams-1(RNAi)* triggers pronounced metabolic remodeling with increased PC, triglycerides, and polyamines alongside decreased mitochondrial metabolites, whereas *sams-4* knockdown shows minimal changes. Furthermore, loss of *sams-1* promotes mitochondrial fission, a phenotype reversible by dietary choline and recapitulated by RNAi targeting PC biosynthetic enzymes, underscoring the key role of SAMS-1–driven PC production. Consistently, *sams-1* deficiency is associated with downregulation of mitochondrial genes, reduced mitochondrial protein abundance, and diminished methylation of mitochondrial proteins during aging, with enhanced mitophagy. This further suggesting that increased mitochondrial turnover contributes to improved stress resistance and longevity. Collectively, these findings position SAMS-1 as a pivotal mediator at the intersection of metabolism and signaling, offering novel mechanistic insights into mitochondrial function, stress and aging.

970C Modeling alternating hemiplegia of childhood in *C. elegans*

Diana Wall[1,2,3], Jeremy Lins[2,3], Roza Khalifa[2,3], Sienna Partipillo[2,3], Anne Hart[2,31]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, [2]Department of Neuroscience, Brown University, [3]Robert J. & Nancy D. Carney Institute for Brain Science, Brown University

Alternating hemiplegia of childhood (AHC) is a rare neurological disease caused by dominant, de novo, missense mutations in ATP1A3 (Heinzen *et al*, 2012). ATP1A3 is the catalytic α3 subunit of a Na+, K+ ATPase that is predominantly expressed in excitable cells. In response to cellular depolarization, this Na+, K+ ATPase plays a critical role in restoring resting membrane potential. However, it is unclear how AHC missense mutations in ATP1A3 disrupt cellular function.

C. elegans EAT-6 is orthologous to human ATP1A3 across the entire length of the proteins (72% identity). *eat-6* was originally identified in an EMS screen for animals with pharyngeal pumping defects, and was later classified as a Na+, K+ ATPase (Avery, 1993; Davis *et al*, 1995). Complete loss of *eat-6* function is lethal, while heterozygotes have disrupted neuromuscular junction (NMJ) function.

Using CRISPR/Cas9-based editing of *eat-6*, we created the first *C. elegans* models of AHC. We have modeled four AHC patient alleles which include the most common and most severe mutations: D801N, E815K, L839P, and G947R. Homozygous AHC model animals are early embryonic or early larval lethal. Based on response to aldicarb, heterozygous model animals have dominant neuromuscular junction (NMJ) defects. This is in contrast to heterozygous *eat-6* null animals, which suggests AHC model animal NMJ defects may be caused by a mechanism beyond loss of ATPase activity.

I plan to use the *C. elegans* models of AHC to investigate how missense mutations in EAT-6 perturb NMJ function on a cellular and molecular level. In addition, we are developing strategies to efficiently conduct a drug or genetic screen with these model animals. These approaches will teach us more about how *eat-6* functions and can be applied to understanding how mutations in ATP1A3 may lead to disease in humans.

971C Mechanical stress modulates the formation of large extracellular vesicles from proteostressed touch neurons

Guoqiang Wang, Lilian Zhou, Emily Friedman, Monica Driscoll Department of Molecular Biology and Biochemistry, Rutgers University

Aging is associated with mitochondrial dysfunction and a loss of proteostasis. Increasing evidence suggests that cells can mitigate functional crises by clearing their contents via the release of extracellular vesicles containing damaged mitochondria and protein aggregates, which can be substrates for non-autonomous degradation.

We previously reported that proteostressed touch neurons can release a large membrane-surrounded extracellular vesicle, termed an exopher, to enhance neuronal function. Given that aggregate transfer from disease to non-disease cells promotes human neurodegenerative disease progression, understanding the mechanisms underlying exopher formation should be critical for advancing fundamental understanding of neuronal health and disease; and possibly inspire therapeutic design.

Our recent work demonstrated that mechanical force from neighboring tissues is crucial in triggering exopher production in young adult *C. elegans* touch neurons. Based on these findings, we hypothesize that a mechanical sensing network is essential for initiating exopher formation.

The cellular skeleton—including the extracellular matrix, cytoskeleton, and nucleoskeleton—is fundamental for sensing mechanical forces and reacting to mechanical stress. Mechanosensitive channels anchor to the extracellular matrix on the outer surface of the plasma membrane and the cytoskeleton on the inner surface. Mechanical stretching of these cellular structures leads to the opening of mechanosensitive channels. By eliminating genes encoding these channels, we found that TRPA-2 and PEZO-1 are required for efficient exopher induction in touch neurons. Notably, most mechanosensitive channels mediate Ca^{2+} influx upon activation. Our time-lapse Ca^{2+} imaging of touch neuron GCaMP3 revealed a significant Ca^{2+} spike that precedes exopher formation, suggesting that the process involves mechanosensitive channel activation and Ca^{2+} influx.

Spectrin, a key cytoskeleton component, maintains plasma membrane stability and facilitates mechanical signal transduction by forming an elastic meshwork beneath the membrane and linking it to the actin network. We found that mutations in the α-spectrin-encoding gene *spc-1* or the β-spectrin-encoding gene *unc-70* significantly increase exopher formation in touch neurons. This suggests that spectrin-mediated resilience to mechanical stress suppresses exopher formation.

Interestingly, Ca^{2+} binding to the EF-hand domains of α-spectrin can destabilize the interaction between the actin network and the spectrin meshwork. We will present our findings on mechanical signaling in exopher production, focused on the interplay between mechanosensitive channels, Ca^{2+} signaling, and the spectrin cytoskeleton.

972C Regulation of the Intracellular Pathogen Response in *Caenorhabditis elegans* by Chromatin Remodelers

Paaramitha Warushavithana, Vladimir Lazetic Biological Sciences, The George Washington University

Chromatin remodelers play a crucial role in regulating innate immune responses across eukaryotes, yet their role in immune activation remains unclear. Caenorhabditis elegans serves as a powerful model for understanding these responses. Specifically, C. elegans elicits the Intracellular Pathogen Response (IPR), a transcriptional program triggered by pathogens, proteostasis disruptions, and heat stress. The IPR shares similarities with the antiviral IFN-I response of mammals. The IPR can be induced by multiple stimuli, including two obligate intracellular pathogens, the fungal species of the Microsporidia family Nematocida parisii, and the positive-sense single-stranded RNA Orsay virus. While transcriptional responses are essential for immunity, constitutive and uncontrolled activation can impair growth. Thus, epigenetic modifications and chromatin remodeling are crucial for regulating immune gene expression both spatially and temporally. In our recent study, we found that loss of the Synthetic Multivulva (SynMuv) gene lin-15B causes constitutive IPR activation and enhances resistance and tolerance against intracellular pathogens. SynMuv genes are essential for development, and some functionally antagonize the H3K36 methyltransferase MES-4 in germline development. Our study shows that this antagonism extends beyond development and plays a role in innate immunity, as the loss of mes-4 suppresses IPR gene expression in lin-15B mutants. Our preliminary data suggest that the loss of mes-4 increases susceptibility to microsporidia and Orsay virus infections independently of SynMuv genes. However, since viral infection can activate the IPR independently of MES-4, other chromatin regulators may regulate IPR activation. To uncover other chromatin remodelers involved in immunity, we performed a targeted RNAi screen of known C. elegans chromatin remodelers or mammalian homologs. Using lin-15B mutants expressing a fluorescent transcriptional IPR reporter, we identified MRG-1 and ISW-1 as IPR activators. MRG-1 is a conserved chromodomain protein that interacts with histone deacetylase and methyltransferase complexes and is essential for germline cell proliferation. ISW-1 is an ATP-dependent chromatin remodeler, likely part of the Nucleosome Remodeling Factor (NURF) complex, and regulates cell fate determination. Together, our findings suggest that chromatin remodelers whose primary role is in development may regulate the innate immunity to provide pathogen resistance.

973C Unraveling Extended Presynaptic Maintenance: Lesson in Longevity from *Caenorhabditis elegans* Dauer Diapause State

Sherlyn P Wijaya, Claire E Richardson Laboratory of Genetics, University of Wisconsin-Madison

Neurons, which primarily develop during embryogenesis, show signs of functional decline with age. In humans, this likely contributes to neurodegenerative diseases like Alzheimer's and Parkinson's. However, Caenorhabditis elegans presents an unusual case, through its dauer diapause state, wherein neuron functions persist despite chronological aging. We aim to exploit this state to uncover the genetic and cellular mechanisms that support this prolonged neuronal maintenance. We focus on the presynapse because it poses special challenges for maintenance due to its distance from the neuron's cell body and its high metabolic demand. Our approach was to first characterize the effects of chronological aging on the dauer larvae's presynaptic cell biology, comparing these findings with changes observed in non-dauer animals with age. Next, we are using a candidate gene approach to identify which of the dauer-promoting transcription factors plays a role in extending presynaptic maintenance, and we further aim to isolate the gene's presynaptic maintenance phenotype from its other dauer-related effects. This work builds on our previous research that discovered how to cell-intrinsically suspend neurite growth in non-dauer animals through a genetic manipulation we termed "dauerization." Here, we aim to achieve a similar "dauerization" of the presynapse to prevent or slow down aging. By understanding the mechanisms of presynaptic maintenance, this work will shed light on the causes of presynaptic decline in aging.

974C Processing and *in situ* detection of TIG-2/TIG-3 in *Caenorhabditis elegans*, an unprecedented BMP to TGF-β/Activin cross-subfamily heterodimer candidate

Zachary Wing[1], Katerina K Yamamoto[1,2], Cathy Savage-Dunn[1,21]Department of Biology, Queens College, City University of New York, [2]PhD Program in Biology, The Graduate Center, City University of New York

The Transforming Growth Factor beta (TGF-β) superfamily, most broadly divided into the bone morphogenetic protein (BMP) and the TGF-β/Activin subfamilies, are secreted dimeric ligands that coordinate a vast range of cellular processes and developmental functions context-dependently. Research has focused predominantly on homodimeric ligands, although the biological function of several notable heterodimers, all intra-subfamily cases, have been demonstrated. Surprisingly, genetic studies in our lab have unveiled a potential cross-subfamily heterodimer candidate, TIG-2(BMP-like)/TIG-3(Activin-like), in the *C. elegans* innate immune response to the bacterial pathogen *Photorhabdus luminescens*.

TGF-β signaling ligands are canonically activated following furin cleavage. TIG-2 has at least two furin sites, suggestive of sequential cleavage. Uniquely, TIG-3 has a single furin site in an atypically upstream location, which is less optimal for ligand:receptor complex assembly according to structural modeling. Interestingly, RNA-seq data of wild-type worms exposed to pathogen shows an upregulation of *nas-11*, a member of the astacin family of metalloproteases. TIG-3 contains a predicted astacin cleavage site that generates a mature TIG-2/TIG-3 heterodimer predicted to bind the receptor complex more optimally. Furthermore, structural modeling predicts the association of NAS-11 to pro-TIG-3 when dimerizing with mature TIG-2. Therefore, a proposed model is prodomain-mediated heterodimerization of TIG-2/TIG-3 in the endoplasmic reticulum, followed by furin cleavage in the Golgi apparatus, with the ultimate bioactivation resulting from astacin cleavage of TIG-3 in the extracellular space.

We will implement a cleavage-site independent strategy to spatiotemporally detect the heterodimer in a living animal using a computationally designed, genetically encoded sensor. The existence of cross-subfamily heterodimers, a phenomenon unlikely to be exclusive to invertebrates due to the highly conserved nature of TGF-β signaling, dramatically broadens the current perspective on combinatorial signaling activity.

975C Programmed cell death throughout life influences the longevity of a defective mitochondrial mutant in *C. elegans*

Sumino Yanase[1], Rea Yamaguchi[2], Kayo Yasuda[3], Naoaki Ishii[31]Daito Bunka University, [2]School of Sports & Health Science, Daito Bunka University, [3]School of Health Studies, Tokai University

In the nematode *Caenorhabditis elegans* (*C. elegans*), the *mev-1* gene encodes a large subunit of the enzyme succinate dehydrogenase cytochrome *b*, which is a component of complex II in the mitochondrial electron transport chain. Mutation of the *mev-1* gene leads to an increase in mitochondrial oxidative stress, thereby inducing abnormal apoptosis in embryonic development and consequently shortening the lifespan. In previous reports, a mutation in the *ced-3* gene encoding an ortholog of mammalian caspases reduced the excessive embryonic apoptosis and recovered the lifespan of the *mev-1* mutant. Here we report the difference between early developmental temporary knockdown and continuous knockdown of the *ced-3* gene in the *mev-1* mutant. We found that CED-3/caspase is essential to not only the abnormal developmental apoptosis in the *mev-1* mutant, but also the excessive mitochondrial dysfunction during aging. These findings indicate that CED-3/caspase has multiple functions in somatic cells.

976C Biosynthesis of modular signaling molecules from *Pristionchus pacificus*

Pei Zhang Beijing Normal University

The model nematode *Pristionchus pacificus* produces four types of complex pheromones named ascarosides named UBAS, DASC, NPAR, and PASC, which are built from various intermediates originating from primary metabolic pathways. However, the exact biosynthetic pathways resulting in the enormous complexity of these modular signaling molecules remain enigmatic. We have previously identified a carboxylesterase *Ppa*-UAR-1 for the biosynthesis of UBAS, enabling the attachment of ureidoisobutyric acid at the 4'-position of simple ascarosides. Here, we additionally report three new carboxylesterases *Ppa*-UAR-5, *Ppa*-UAR-6 and *Ppa*-UAR-12 from *P. pacificus*. *Ppa*-UAR-5 functions downstream of *Ppa*-UAR-1 to furnish the biosynthesis of ubas#1 and ubas#2, whereas *Ppa*-UAR-12 specifically links two ascr#1 at the 4'-position to synthesize dasc#1. Finally, *Ppa*-UAR-6 is essential for the biosynthesis of npar#1-3 and part#9. The expression pattern of *Ppa-uar-6* and *Ppa-uar-12* in intestinal and epidermal cells suggest pheromone biosynthesis to be restricted to specific tissues. These findings indicate that the expansion and functional diversification of carboxylesterases played a crucial role for the evolution of complex pheromones in nematodes.

Keywords: *Pristionchus pacificus*, ascaroside, biosynthesis, *Caenorhabditis elegans*, modular signaling metabolites, pheromone