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ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

1 **Deciphering mechanisms of centriole elimination during oogenesis** Alexander Woglar, Keshav Jah, Fabian Schneider, Marie Pierron, Coralie BussoEPFL

Centrioles are nine-fold symmetrical microtubule-based organelles that template the formation of cilia and flagella, and form the core of the centrosome that organizes cytoplasmic microtubules, including during cell division. The number of centrioles must be tightly controlled: like chromosomes, each of the two centrioles present early in the cell cycle is licensed to seed the formation of a new centriole once, and only once during S-phase. However, during reproduction, centriole numbers must be controlled differently. Here, sperm and egg cells fuse to form a single cell. Here, the sperm contributes two centrioles, while centrioles are eliminated during oogenesis, thus avoiding doubling the number of centrioles with each generation.

We present mechanistic insights into this evolutionarily conserved and thus far enigmatic process by employing the gonad of *C. elegans* as an optimal model system, using ultrastructure expansion coupled with STED microscopy, electron microscopy and tomography, live-imaging and novel genetic and acute pharmacological inhibition methods. We find that oogenesis centriole elimination begins when nuclei enter late meiotic prophase I (late pachytene) and is characterized by several ultrastructural and compositional changes. First, the microtubule-binding and -bundling factor SAS-1 is eliminated from centrioles at that stage during oogenesis. This is followed by widening of the centriole and progressive decoration of the core centriolar components microtubules and SAS-4 with ubiquitin. Thereafter, the proteasome becomes enriched at centrioles, which coincides with loss of the ubiquitinated centriolar microtubules and SAS-4, followed by a complete loss of centriolar integrity. Importantly, these processes happen prematurely in *sas-1* mutant animals.

We propose that centriole elimination during oogenesis involves differential access of the proteasome to the centriole, which is controlled by an alteration in centriolar architecture imparted by the loss of SAS-1.

2 **Determining the mechanism of Kinesin-1 dependent translocation of the meiotic spindle to the cortex** Alma Martinez Peraza[1], Francis J McNally[21]University of California, Davis - Davis, CA, [2]MCB, 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* the prophase nucleus migrates to the oocyte cortex and the metaphase spindle moves further toward the cortex, both in a kinesin-1 dependent manner. In contrast, yolk granules, mitochondria and kinesin-1 are packed inward, away from the cortex in a kinesin-dependent manner. The kinesin-dependent inward packing of yolk granules and mitochondria suggests the existence of microtubules with minus ends at the cortex and plus ends extending inward. Thus, the mechanism of outward translocation of the spindle has remained a mystery. 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, duIs1, that is silenced in the germline. Time-lapse imaging of *unc-116(gk5722) duIs1* worms revealed a stronger phenotype than previously reported for viable alleles or RNAi depletions, with the meiotic spindle positioned in the center of the embryo. Kinesin-1 has been reported to bind to multiple different cargoes through its C-terminal tail domain. To identify the direct cargo of kinesin that mediates movement of the meiotic spindle to the cortex, we are taking an optogenetic approach to couple tailless kinesin-1 directly to ER, nuclear envelope, mitochondria, or yolk granules to determine if this restores spindle translocation in *unc-116(gk5722) duIs1* embryos. K420 is the first 420 aa of UNC-116 which does not include the cargo-binding tail. TMCO-1 is an integral membrane protein of the ER. iLID and SSPB bind when illuminated. Attaching K420::mKate::iLID tailless kinesin specifically to TMCO-1::GFP::SSPB labeled ER has restored the localization of the meiotic spindle to the cortex in an *unc-116(gk5722) duIs1* background in 6/6 time-lapse sequences. This result suggests that the ER, which envelopes the meiotic spindle after nuclear envelope breakdown, may be the direct cargo of kinesin-1 in meiotic spindle translocation. TMCO-1 is in the nuclear envelope as well as the ER whereas the nuclear pore protein NPP-24 is only in the nuclear envelope. To determine whether kinesin-1 acts before or after nuclear envelope breakdown, we are attempting the same experiment with NPP-24::GFP::SSPB.

3 **Repurposing the Chromosome-Microtubule Coupling Machinery as a "Tuner" of Actin for Dendritic Branching.** Dhanya Cheerambathur[1], Mattie Green[2], Henrique Alves Domingos[2], Vasilis Ouzounidis[21]School of Biological Sciences, University of Edinburgh, [2]University of Edinburgh

Dendrite branching is an essential process for building complex nervous systems. A neuron's dendritic patterns govern the number, distribution, and integration of inputs. Though significant progress has been made in understanding the signalling pathways that pattern the dendrite, little is known about the intrinsic mechanisms involved in sculpting the branches. The actin & microtubule cytoskeleton are critical to provide structure and exert force during dendrite branching. Our study reveals an unexpected role for the kinetochore, the chromosome-microtubule machinery, in shaping the dendrites of the mechanosensory neuron, PVD in *C. elegans*. The kinetochore is a highly conserved multiprotein complex whose canonical function is to connect chromosomes to microtubules during cell division. Kinetochore proteins are enriched in the PVD dendrites where they associate

with the endosomal structures and are essential for establishing the dendritic pattern of PVD independent of its cell division function. Degradation of kinetochore proteins during PVD development results in dendrite branch fusion and overexpression of kinetochore leads to hypo-branching. Surprisingly, microtubule dynamics remain unchanged in the absence of kinetochores, but F-actin dynamics is altered during dendrite branching. We show that kinetochore proteins modulate F-actin polymerization mediated by the Rac GTPases. Thus, our work suggests that kinetochore proteins act as a "tuner" of actin polymerization to control dendrite patterning. Overall, these findings reveal an unexpected architect in dendritic branching and provide insight into crosstalk between microtubules and actin-based structures that remodel dendrites.

4 **Endocytosis in the axon initial segment maintains neuronal polarity** Kelsie Eichel[1], Vivek Belapurkar[2], Caitlin Taylor[1], David Perrais[2], Kang Shen[11]Stanford University, [2]University of Bordeaux

Neurons are highly polarized cells with distinct axonal and dendritic domains that send and receive signals, respectively. To achieve this polarization, neurons extensively use membrane trafficking mechanisms to compartmentalize a vast and diverse repertoire of proteins to each domain. The axon initial segment (AIS) is a critical region separating these domains and is known to function in neuronal polarity as a selective filter for intracellular vesicle trafficking and a diffusion barrier on the plasma membrane. However, it remains unclear how the AIS maintains stringent polarity over the decades-long lifetime of the neuron. We identified a novel, active function of the AIS in neuronal polarity that is conserved from *C. elegans* to humans. Using the extremely morphologically polarized *C. elegans* PVD sensory neuron, we find that dendritically and axonally polarized transmembrane proteins are recognized by endocytic machinery in the AIS, robustly endocytosed, and targeted to late endosomes for degradation. Engineering receptor interaction with AIS master organizer, ankyrinG, antagonizes receptor endocytosis in the AIS, causes receptor accumulation in the AIS, and leads to polarity deficits with subsequent morphological and behavioral defects. We then extended these findings to cultured rodent and induced human neurons. Thus, endocytosis is a broadly used, active mechanism to capture and remove axonal and dendritic proteins from the AIS to maintain their compartmentalization. Our results reveal a conserved endocytic clearance mechanism in the AIS that is essential for neuronal polarity and define a framework for understanding AIS endocytosis. This endocytic clearance mechanism works in concert with known polarity mechanisms of the AIS to maintain polarity over the long lifespan of the neuron. Through the study of neurons, one of the most polarized cell types, the present results reveal a mechanism by which cells can achieve strict compartmentalization even along a contiguous membrane region.

5 **A brain-to-gonad-to-embryo adrenergic signaling relay controls intergenerational transfer of temporal learning ability** Eugene L.Q. Lee, H. Robert HorvitzHHMI, Dept. Biology, MIT

Animals recognize patterns in the timing of stimuli in their environment, associate the relevant events, and when subsequent similarly timed events reoccur respond appropriately to optimize survival. How such temporal processing events are coordinated at cellular and molecular levels across tissues to produce adaptive behavior is unclear. We show that *C. elegans* is sensitive to the temporal patterning of sensory stimuli. Worms learn to associate a neutral odor stimulus with a noxious aversive light stimulus specifically when these stimuli are paired in an ordered temporal pattern in which the odor is predictive of subsequent light exposure. Notably, *C. elegans* is capable of trace-conditioning – a form of learning in which there is a delay between the presentation of a neutral (odor) stimulus and the presentation of a noxious (light) stimulus - showing that worms have the ability to detect and distinguish timing durations. We found that adrenergic signaling alters the temporal processing of the timed duration between associated stimuli. Worms defective in the synthesis of the adrenergic biogenic amines tyramine and/or octopamine (i.e., *tdc-1* and *tbh-1* mutant worms) exhibit enhanced trace-responses compared to wild-type worms and are able to associate stimuli across longer trace-delay periods. Remarkably, we discovered that trained parental worms produce progeny with enhanced sensitivity to the temporal patterns of trace-conditioning. Transmission of the experiential signal is dependent on the timed ordering of stimuli, as worms exposed to equal levels but randomly ordered patterns of learning stimuli do not exhibit such intergenerational effects. This intergenerational inheritance is flexibly coordinated by a brain-to-gonad-to-embryo communication axis that also acts through adrenergic signaling: inhibiting parental adrenergic output from either the RIM/RIC neurons or the somatic gonad abolishes inheritance of temporal sensitivities. Our results demonstrate that adrenergic signaling functions in a central inter-organ communication system for tuning temporal processing within and across generations.

6 **Nematode extracellular protein interactome expands connections between signaling receptors and ligands** Viola I Nawrocka[1,2,3], Shouqiang Cheng[1,2,3], Matthew Rosen[1,2,3], Elena Cortés[1,2,3], Elana Baltrusaitis[1,2,3], Zainab Aziz[1,2,3], István Kovács[4], Engin Özkan[1,2,31]Biochemistry & Molecular Biology, The University of Chicago, [2]Institute for Neuroscience, The University of Chicago, [3]Institute for Biophysical Dynamics, The University of Chicago, [4]Physics and Astronomy, Northwestern University

Multicellular complexity in metazoans was accompanied by the emergence of new classes of cell surface receptors and secreted ligands, which have taken on functions conserved through animal development and physiology. New genomic data sets and bioinformatic tools have allowed us to identify extracellular proteomes, and study their interactions using high-throughput methods. The nematode *C. elegans* is a favorable model to study cell surface interactomes, given the highly defined and stereotyped

cell types and intercellular contacts, including its entire connectome. Here we report the largest extracellular interactome for an invertebrate, most of which are novel interactions despite recently released datasets for flies and humans, as our collection contains a larger group of protein families. We report novel interactions for all four major axon guidance pathways, including those that connect three of the pathways by physical interactions in the extracellular space. In addition, we show that an immunoglobulin superfamily of proteins known to guide and maintain axon locations are secreted co-receptors for the expanded insulin family in *C. elegans*, and may antagonize insulin action by forcing insulin receptors into an inactive conformation. We also report novel interactions that allow us to define classes of cytokine-like secreted proteins binding to signaling receptors in the RTK class, which may extend use of nematodes as a model organism for studying cytokine and growth-factor-mediated vertebrate functions. Finally, our dataset provides new evidence and insights into how extracellular interactions may help define connectomes, including novel interactions for previously known synapse targeting receptors.

7 **Caenorhabditis Genetics Center** Aric L Daul[1], Julie Knott[1], Liz Fox[1], Kat Piloto[2], Adam Grazzini[2], Celine Smith[2], Ann E Rougvie[21]GCD, Univ of Minnesota, [2]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, and genetic tool strains for various applications such as inducible gene expression. A searchable list of our nearly 25,000 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!

8 **Critical update on WormBase** Paul W Sternberg[1], WormBase W Consortium[2], WormBase W Consortium[31]California Institute of Technology, [2]European Bioinformatics Institute, [3]Ontario Institute for Cancer Research

We will discuss the state of WormBase and how we will transition services to the Alliance of Genome Resources (AllianceGenome.org) over the next two years. This talk will outline the critical aspects most *C. elegans* (and other nematode) researchers will most want to know. WormBase will still curate information (small and large scale) from published papers and develop displays for unique datasets; the Alliance will provide the software infrastructure for WormBase as well as an increasing number of model organism knowledgebases. The Alliance site is already the best place to start with any worm, fly, yeast, or human gene to find much basic information and orthology. However, WormBase.org now provides way more details useful for planning experiments and richer data. This information will gradually be moved to the WormBase area of the Alliance (alliancegenome.org/members/wormbase). WormBase ParaSite will continue to handle the multitude of worm genomes. We hope that the Alliance will be able to handle many genomes and associated rich biological data in the future.

Our funders now count citations to papers describing WormBase; the current citation is: Davis et al. Genetics 2022 (PMID: 35134929). Please cite this paper if you use information from WormBase in the planning, execution or interpretation of your data described in the publication!

WormBase was selected as one of 37 Core Global Biodata Resources.

9 **Coupling of growth rate and developmental tempo reduces body size heterogeneity *in C. elegans*** Klement Stojanovski[1], Helge Grosshans[2], Benjamin Towbin[11]University of Bern, [2]Friedrich Miescher Institute for Biomedical Research

Animals increase by orders of magnitude in volume during development. Therefore, small variations in growth rates among individuals could amplify to a large heterogeneity in size. By live imaging of *C. elegans*, we show that amplification of size heterogeneity is prevented by an inverse coupling of the volume growth rate to the duration of larval stages and does not involve strict size thresholds for larval moulting. We perturb this coupling by changing the developmental tempo through manipulation of a transcriptional oscillator that controls the duration of larval development. As predicted by a mathematical model, this perturbation alters the body volume. Model analysis shows that an inverse relation between the period length and the growth rate is an intrinsic property of genetic oscillators and can occur independently of additional complex regulation. This property of genetic oscillators suggests a parsimonious mechanism that counteracts the amplification of size differences among individuals during development.

10 **Innexins and Enac channels coordinate muscle activity in embryos to stimulate body elongation** FloRA LLENSE, Teresa FERRARRO, Hanra Song, Coline Varigault, Michel LabouesseLBD, Sorbonne Université, IBPS, CNRS UMR7622

Body axis elongation represents a fundamental morphogenetic process in development, which involves cell shape changes powered by mechanical forces. if local and tissue scale forces and their participation are well described, little is known on how two mechanically coupled tissues coordinate their behavior in order to build a full organism. *C.elegans* elongation provides a perfect model system to study this question. During elongation, cyclic forces resulting from muscle contractions leads to adherent junctions and actin cytoskeleton remodeling in the epidermis allowing progressive embryo lengthening. Previous studies have clearly identified the different players involved at the hypodermis cell but we still miss a full understanding of how muscles cell activity is controlled and how the 4 muscle quadrants coordinate their activity in order to allow the incremental lengthening of the embryo. Using a Calcium sensor to monitor muscle activity during elongation, we identified two cells in each muscle quadrant that act as pacemaker and control muscle activity within each quadrant. Remarkably, ablation of these two cells abolished muscle contraction along the anterior posterior axis leading to embryo elongation arrest at the 2-fold stage. To identify new genes involved in muscle activity, we performed a RNAi screen targeting two class of proteins: the innexins and the DEG/ENAC channels. Among them, we found that two ENAC channels and two innexins control muscle activity and are required for normal embryonic elongation. One of these innexins is specifically expressed in the intestinal cell. We are currently investigating in more details how these two innexins impact muscle cell activity and the overall lengthening of the embryo. Altogether our data provide a new understanding how embryonic body wall muscle coordinated their activity and how multiple mechanically coupled tissues ensure the proper morphogenesis.

11 **The *C. elegans* "hibernation"** Yanwu Guo, Rafal CioskDepartment of Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo

We and others have shown that *C. elegans* can survive spells of severe cold, and their natural cold resistance can be improved through genetic manipulations. In one example, removing the otherwise non-essential transcription factor ETS-4 markedly improves cold resistance. In ETS-4(-) animals, two other transcription factors, DAF-16/FoxO and PQM-1, jointly promote the expression of a ferritin variant, *ftn-1*. In turn, FTN-1 promotes cold survival by detoxifying cold-induced, ROS-generating iron species. Remarkably, the cold survival-promoting role of ferritin is conserved in mammalian cells, demonstrating that C. elegans can be used as a model to uncover conserved pathways promoting cold survival with exciting biomedical implications [1].

The induction of specific genes promoting cold survival is seemingly inconsistent with observations from other models, where profound cooling induces a global reduction of translation. Thus, we have examined it also in *C. elegans* and found that, like in other models, cold results in a drastic reduction of translation. These observations suggest the existence of dedicated mechanism(s) promoting the expression of specific genes in cold-challenged animals. Employing gene expression profiling and gene-specific reporters, our results suggest that the specificity is achieved chiefly at the transcription level. Our current studies aim at identifying the underlying molecular mechanisms.

[1] Nat Commun 13, 4883 (2022). https://doi.org/10.1038/s41467-022-32500-z

12 **How does starvation promote cellular plasticity during Y-to-PDA transdifferentiation?** Julien Lambert[1], Jarriault Sophie[21]Department of Development and Stem Cells, IGBMC, [2]IGBMC

During development, cells switch off a large share of the genes encoded in their genome, while maintaining or starting the expression of others. This process of genome specialization is one of the keys that enable the generation of a wide range of diverse differentiated cells from a single genome. Mechanisms such as repression of genes associated with alternative fates, or involved in the cell cycle, are required both for the establishment of these specific rewiring, but also to their maintenance, in order to safeguard differentiated identities. However, little is known about their relevance in cells that change their identity, neither whether they are affected by environmental and physiological cues.

The Y-to-PDA transdifferentiation is a prominent example of cellular plasticity from a differentiated cell. The first step of this process consists in the erasure of the initial identity and the loss of Y epithelial features. 2 parallel pathways are key for this initiation step. On the one hand, a cassette of conserved plasticity factors including EGL-27 triggers the switch, while on the other hand, we found that the factor LIN-15A inhibits identity safeguarding mechanisms, such as the DREAM complex in Y-to-PDA. Importantly, our data indicate that EGL-27 (ortholog of human histone deacetylase MTA1) and LIN-15A are the sole factors required for Td initiation linked to chromatin remodeling.

Furthermore, we have found that the defects in Td initiation due to the loss of either of these two factors can be suppressed upon starvation, slow growth conditions or under specific environmental conditions such as different food sources. Our results

demonstrate that starvation specifically increases the plasticity of the Y cell in *lin-15A* or *egl-27* mutants (beyond its impact on growth). Moreover, we have found that starvation and slow growth impact cellular plasticity through different mechanisms. Our data point to lower general metabolic activity rather than slow growth itself as the plasticity-increasing factor. On the other hand, the starvation signal is mediated and integrated specifically by the Insulin/IGF-1 Signaling. We will present our latest data on how the information of starvation is transmitted to a plastic cell. We will also propose a model for how, under conditions of low metabolic activity, cells may invest less resources into genome specialization, therefore making the chromatin remodeling activities of LIN-15A and EGL-27 dispensable for Td.

13 **Pulling or Pushing? Revisiting the mechanics of *C. elegans* gonad morphogenesis** Priti Agarwal[1], Tom Shemesh[2], Ronen Zaidel-Bar[31]Cell and Developmental Biology, Tel Aviv University, [2]Technion - Israel Institute of Technology, [3]Tel Aviv University

Organ morphology is critical for its function. Biochemical signals along with mechanical cues influence multiple cell and tissue behaviors to shape an organ during development. Here, we use *C. elegans* as a model to study the mechanical basis of gonad development. The *C. elegans* gonad has two symmetrical U-shaped arms, each with a single somatic cell known as Distal Tip Cell (DTC) at its tip. The DTC is thought to function as a leader cell guiding multiple follower germ cells to form the U-shaped architecture. The gonad initially elongates on the ventral surface away from the midbody, then initiates a U-turn towards the dorsal surface, finally moving back towards the midbody. However, the mechanism of DTC migration has remained elusive. Here, we used live-imaging, laser ablations, and DTC-specific genetic manipulations, to show that the gonad does not elongates by a pulling force from the leader cell, but rather due to a pushing force generated by the proliferating germ cells, which are confined by a basement membrane behind the DTC. Local release of matrix-degrading metalloproteases by the DTC determines the direction of gonad elongation. A qualitative physical model we created predicted that differential cell-matrix adhesion could create torque that would drive gonad turning, and we provide experimental evidence to support this idea. Integrin-mediated adhesion is enriched on the dorsal side of the DTC specifically during the U-turn, and genetic perturbations that interfere with adhesion polarity lead to turning defects, including no turn and reversal of turn direction. Taken together, our study provides novel mechanistic insight into organ morphogenesis, i.e., directed invasion assisted by proliferative pressure and asymmetric adhesion, which may also be relevant for other developmental systems as well as solid tumor metastasis.

14 **EXC-4 CLICs into signaling: defining the conserved function of chloride intracellular channels in Gα-Rho/Rac signaling** Jordan Jesse[1,2], Anthony Arena[1,2], Julianna Escudero[1], Daniel Shaye[11]Physiology and Biophysics, University of Illinois at Chicago - College of Medicine, [2]Graduate Education in Biomedical Sciences, University of Illinois at Chicago - College of Medicine

Chloride intracellular channels (CLICs) are an enigmatic family of proteins whose physiological and molecular functions remain mysterious. A conserved role for CLICs in tubulogenesis was suggested by the fact that *C. elegans* EXC-4/CLIC regulates tubulogenesis of the excretory canal (ExCa), a unicellular tube, while vertebrate CLIC1 and CLIC4 regulate angiogenic behaviors of human umbilical vein endothelial cell (HUVEC) *in vitro* and murine vascular development *in vivo*. CLIC1 and CLIC4 are required G-protein-coupled receptor (GPCR)-heterotrimeric G protein (Gαβγ)-induced RhoA and Rac1 activation in HUVEC (Mao, *et al.*, Kleinjan, *et al.*), and this function is conserved, as we recently showed that EXC-4 genetically interacts with Gα-Rho/Rac signaling to promote ExCa outgrowth (Arena, *et al.*). We identified a conserved C-terminal motif required for EXC-4-regulated ExCa outgrowth and hypothesize that this motif mediates a functional interaction between EXC-4/CLICs and Gα-Rho/Rac signaling. Notably, human CLICs display context-dependent functions in HUVEC, as CLIC1 is required for RhoA and Rac1 activation, while CLIC4 is only required for Rac1 activation, in response to the GPCR ligand S1P (Mao, *et al.*). Conversely, only CLIC4 is required for RhoA activation in response to the ligand thrombin (Kleinjan, *et. al*). This leads us to further hypothesize that molecular features of EXC-4/CLIC C-termini provide specificity to function in Rho/Rac signaling. To test these hypotheses we are undertaking structure-function studies in *C. elegans* and HUVEC to define shared and unique motifs in EXC-4, CLIC1, and CLIC4 required for function. We also immunoprecipitated flourescently tagged wildtype and C-terminal mutant EXC-4, specifically expressed in the ExCa, and used mass spectroscopy to identify potential binding partners that require the conserved C-terminus motif for interaction. Among the putative interactors we identified several actin-regulating proteins and members of the chaperonin-containing T-complex, and we are currently investigating the function of these putative interactors in ExCa outgrowth. We expect these studies will allow us to define how EXC-4/CLICs interface with Rho/Rac signaling during tubulogenesis and angiogenesis.

Mao Y, Kleinjan ML, Jilishitz I, Swaminathan B, Obinata H, Komarova YA, Bayless KJ, Hla T, Kitajewski JK. CLIC1 and CLIC4 mediate endothelial S1P receptor signaling to facilitate Rac1 and RhoA activity and function (2021)

Arena AF, Escudero J, Shaye DD. A metazoan-specific C-terminal motif in EXC-4 and Gα-Rho/Rac signaling regulate cell outgrowth during tubulogenesis in *C. elegans* (2022)

Kleinjan M, Mao DY, Naiche LA, Joshi J, Gupta A, Jesse J, Shaye D, Mehta D, and Kitajewski JK. CLIC4 regulates endothelial barrier

control by mediating PAR1 signaling via RhoA (*in revision*)

15 ***C. elegans* SMOC-1 interacts with both BMP and glypican to regulate BMP signaling** Melisa S DeGroot, Byron Williams, Timothy Y Chang, Maria L Maas Gamboa, Isabel M Larus, J. Christopher Fromme, Jun LiuMolecular Biology and Genetics, Cornell University

Secreted modular calcium binding (SMOC) proteins are conserved matricellular proteins found in organisms from *C. elegans* to humans. SMOC homologs characteristically contain one or two extracellular calcium (EC) binding domain(s) and one or two thyroglobulin type-1 (TY) domain(s). SMOC proteins in *Drosophila* and *Xenopus* have been found to interact with cell surface heparan sulfate proteoglycans (HSPGs) to exert both positive and negative influences on the conserved bone morphogenetic protein (BMP) signaling pathway. In this study, we used a combination of biochemical, structural modeling, and molecular genetic approaches to dissect the functions of the sole SMOC protein in *C. elegans*. We showed that SMOC-1 binds LON-2/glypican, as well as the mature domain of DBL-1/BMP. Moreover, SMOC-1 can simultaneously bind LON-2/glypican and DBL-1/BMP. The interaction between SMOC-1 and LON-2/glypican is mediated by the EC domain of SMOC-1, while the interaction between SMOC-1 and DBL-1/BMP involves full-length SMOC-1. We further showed that while SMOC-1(EC) is sufficient to promote BMP signaling when overexpressed, both the EC and TY domains are required for SMOC-1 function at the endogenous locus. Finally, when overexpressed, SMOC-1 can promote BMP signaling in the absence of LON-2/glypican. Taken together, our findings led to a model where SMOC-1 functions both negatively in a LON-2-dependent manner and positively in a LON-2-independent manner to regulate BMP signaling. Our work provides a mechanistic basis for how the evolutionarily conserved SMOC proteins regulate BMP signaling.

16 **AMPK regulates a miRNA-based signal that instructs germ cell quiescence through the release of neuronal extracellular vesicles** Chris Wong, Elena Jurczak, Richard RoyBiology, McGill University

Stem cell divisions are particularly interesting from both a cell biological and developmental perspective due to their intrinsic ability to generate progenitor cells and their ability to self-renew. However, despite these interesting aspects of their cell divisions, many stem cells don't divide at all, unless they receive specific signals to proliferate. To understand the genetic pathways that control the dynamics of quiescence and proliferation in stem cells, we use *Caenorhabditis elegans* to investigate potential mechanisms that regulate cell cycle decisions in the germline stem cells.

In adverse conditions, *C. elegans* enter a stress-resistant stage called «dauer» and our work has highlighted the role of AMPK signalling, not only for the survival of the animal during this period, but also for the post-dauer fertility of these animals when they recover. In replete conditions, mutants that lack all AMPK signalling (*aak(0)*) develop like wild-type animals. However, *aak(0)* mutants that transit through the dauer stage emerge completely sterile due to inappropriate chromatin modifications and the associated maladaptive gene expression.

Our recent data indicate that AMPK activity is required in the neurons to activate a cellular trafficking mechanism that results in the loading and movement of extracellular vesicles (EVs) from the neurons to the germ line. Through tissue-specific RNAi analyses, we show that microRNA biosynthesis is required only in the neurons to produce this pro-quiescent signal that acts in the germ line. These microRNAs are then likely packaged into late endosomes/intralumenal vesicles to form multivesicular bodies in the neurons through the activity of Rab and Sid gene products. These multivesicular bodies then fuse with the plasma membrane, secreting the EVs from the neurons. Through biochemical analyses, we show that the microRNA Argonaute proteins are secreted by the neurons inside these EVs. In the germ cells, these EVs are internalized through the kinase activity of the non-receptor tyrosine kinase *sid-3*. Thus, these miRNA-based signals relay a message to adjust germline gene expression in anticipation of a long period of developmental quiescence. Our findings reveal a novel role for AMPK in directing small RNA trafficking into neuronal vesicles to coordinate changes in the germ line that are critical for germ cell integrity and reproductive fitness.

17 **Distinct heparan sulfate modification patterns control proliferation and differentiation of germline stem cells in *Caenorhabditis elegans*** Dayse S da Cunha[1], Andrea dV Carranza[2,3], Sebastian Rojas Villa[1], Antonio Cádiz[3], Kristina Ames[3], Helena B Nader[4], Hannes E Bülow[1,5], Alicia Meléndez[31]Department of Genetics, Albert Einstein College of Medicine, [2]Laboratory of Molecular, Cellular and Genomic Biomedicine, Biomedicina Molecular, Celular y Genómica, Fundación para la Investigación Sanitaria La Fe de Valencia, [3]Department of Biology, Queens College, and Biochemistry Ph.D. Program, The Graduate Center of the City University of New York, [4]Disciplina de Biologia Molecular, Departamento de Bioquímica, Faculdade de Medicina, Universidade Federal de São Paulo, [5]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine

Stem cells are a specialized population of cells with limitless capacity for self-renewal. Often, a stem cell divides asymmetrically to give rise to one daughter that further differentiates to adopt a terminal fate whereas the other daughter maintains the stem cell potential. In addition to signals between individual stem cells, the stem cell population is in association with and governed by cells of the stem cell niche. This tissue, which is in close physical proximity with the dividing stem cells contributes to

maintaining stemness of the daughter cells and coordinates differentiation. The extracellular matrix is important for stem cell biology, but many aspects of ECM function remain elusive. This is also true for the functions of heparan sulfate, an extracellular glycan of great molecular diversity. This diversity stems from complex, non-uniform modifications of the glycan chains. The sugar residues along the chains can be modified by dedicated HS modifying enzymes, which introduce modifications such as sulfation, deacetylation and epimerizations in various positions. Due to these non-uniform modifications, HS chains adopt a domain structure and display an enormous molecular diversity with nearly boundless combinatorial possibilities. We have found that distinct combinations of HS modifications are involved in regulating the homeostasis of *C. elegans* germ line stem cells. Specifically, mutations in certain modification enzymes increase, whereas mutations in genes coding for other modification enzymes decrease the number of stem cells in the proliferative zone of the *C. elegans* adult germline. Intriguingly, these effects may be mediated by regulating several signaling pathways, such as Fibroblast Growth Factor signaling and GLP-1/Notch. At least some of the functions of HS are non-autonomously controlling proliferation of stem cells in the germline. Collectively, these findings suggest that distinct HS modification patterns display differential effects on germline stem cell homeostasis, likely by modulating signaling between the germline niche and the germline stem cells.

18 **DAF-18 prevents oocyte wastage in spermless hermaphrodites through activating calcium signaling and contractility in the spermatheca neck** Jichao DengUniversité du Québec à Trois-Rivières

In *C. elegans* hermaphrodites that lack sperm, DAF-18 is required for oocytes to arrest and accumulate in the proximal gonad. As a result, spermless *daf-18* mutants fail to down-regulate germline stem cell proliferation and continue to produce, and waste, their precious unfertilized oocytes. To begin to address how DAF-18 permits oocyte accumulation in the absence of sperm, we rescued *daf-18* specifically in the germline, gut, neurons, hypodermis and muscles of spermless *daf-18* null mutants. Surprisingly, we found that muscle *daf-18* expression was sufficient to allow unfertilized oocytes to arrest and accumulate in spermless animals, while expression in the other tissues, including the germline, had no effect. While several somatic gonadal cells are contractile muscle-like cells, we could further pinpoint that rescuing *daf-18* specifically in the spermatheca neck, was sufficient to permit oocyte arrest. To accomplish this, we found that DAF-18 cell autonomously promotes Ca^{2+}signaling and contractility in the spermatheca neck to prevent unwanted ovulation events. We further found that this function of DAF-18 required its lipid phosphatase activity, and is thus mediated by PIP_3/PIP_2 levels. While PIP_2 is a substrate for PLC-1 and can thus by itself promote contractility via the IP_3/ITR-1 cascade, we also show that PIP_3 levels also influence cytoplasmic Ca^{2+} influx via the stimulation of AKT-1. Overall, our results demonstrate that DAF-18 promotes contractility in the spermatheca neck to non-autonomously mediate oocyte arrest in the absence of sperm, and allow for their accumulation in the proximal gonad, to ultimately downregulate germline stem cell proliferation. These results provide a new mechanism through which *daf-18*'s human ortholog can act to prevent tumour formation.

19 **A sensory cilium mediates specific neuron-glia attachment** Leland Wexler[1,2], Maxwell Heiman[1,2], Irina Kolotueva[31]Genetics, Boston Childrens Hospital, [2]Harvard Medical School, [3]University of Luasanne

Glial cells form specialized attachments with specific neuronal partners, but how such neuron-glia pairing occurs remains unknown. The URX neuron offers a remarkable example of specificity in neuron-glia pairing. The URX dendrite is positioned in the dorsal sensory bundle of the head and makes a "jump" across the nose tip to attach to a specific glial partner (ILso) in the lateral sensory bundle. Although 36 different glial endings are present at the nose tip, URX recognizes and makes exclusive stereotyped attachments to the lateral ILso glial cell. We found that this specific neuron-glia pairing occurs by a multistep process. First, in comma-stage embryos, the URX dendrite anchors to a glial guidepost, likely the dorsal CEPsh glial cell, such that the dendrite is stretched out to its full length during embryo elongation. Anchoring requires the adhesion protein SAX-7, which acts both in neurons and glia, and the scaffolding protein GRDN-1, which is required only in glia. Mutants that disrupt dendrite anchoring result in severely shortened URX dendrites that fail to reach the nose tip. Second, in pretzel stage embryos, the URX dendrite develops a sensory cilium that jumps across the nose to form the mature attachments to the lateral ILso glial cell. In *daf-19* mutants that do not form cilia, the URX dendrite is full length but remains dorsally positioned and does not attach to the lateral ILso glia, showing that the URX cilium is required for mature glial attachment. To identify mechanisms that control cilia-glia attachment, we used a candidate screen and found that the loss of the conserved SPARC family protein TEST-1 results in loss of the attachment to the lateral ILso glial cell, with URX cilia failing to leave the dorsal bundle. Importantly, vertebrate SPARC family proteins are secreted by glia and regulate adhesion at synapses, suggesting cilia-glia pairing may resemble pairing of dendritic spines and glia at vertebrate synapses. Finally, we used a forward genetic screen to isolate a collection of mutants with defects in URX-ILso attachment, implicating a possible role for glycan modifications in cilia-glia adhesion. Together, our results suggest that, in addition to their canonical role in sensory signaling, cilia can mediate cell-cell adhesion including in the context of specific neuron-glia pairing.

20 **Loss of sensory dendrite cilia is detected by surrounding glia via neuron/glia protein pair DGS-1/ FIG-1** Katherine C Varandas[1], Brianna Hodges[1], Lauren Lubeck[2], Amelia Farinas[3], Yupu Liang[4], Yun Lu[1], Shai Shaham[11]Developmental Genetics, The

Rockefeller University, [2]Biology Graduate Program, Stanford University, [3]Neurosciences Graduate Program, Stanford University, [4]Alexion Pharmaceuticals

Cell structure is critical for cell function. This is particularly evident in the nervous system where neurons and glia take on elaborate shapes and extreme sizes to carry out their functions. An important question is whether and how cell structure is monitored by interacting cells. We addressed this question in the major *C. elegans* sensory organ, the amphid. In the amphid, a glial cell, the AMphid SHeath glia (AMsh), secretes extracellular matrix that surrounds sensory dendrite cilia. We developed a method to monitor AMsh secretion by imaging a GFP-tagged AMsh-secreted matrix protein, VAP-1. We found that VAP-1 accumulates in the amphids of animals with cilia mutations, which cause loss of cilia structure and sensory function. Consistent with this, electron microscopy reveals accumulation of matrix-filled vesicles within AMsh glia and matrix in the extracellular space surrounding sensory dendrite cilia in cilia mutants. Inducible cilia destruction shows that the glial response to cilia loss is acute, as ablating cilia induces AMsh glia matrix accumulation within hours. Additionally, we compared AMsh glia gene expression between wild type and cilia mutants and found extensive transcriptional changes, correlating with increased activity of the conserved glial transcription factor PROS-1, in cilia mutants. PROS-1 controls expression of the AMsh glia secretome and its homologs promote glial differentiation and neuronal repair. We conclude that glia monitor the structure or activity of the dendrites they ensheath and respond by altering their secretion and transcription. To search for molecules involved in signaling from dendrites to glia, we then performed a forward genetic screen in which we isolated a mutant with normal cilia morphology but inappropriate accumulation of AMsh glia matrix. These mutants carry a causative lesion in a gene encoding a 7-transmembrane domain protein, which we named Dendrite-Glia Signaling-1 (DGS-1). DGS-1 is expressed and functions in a subset of sensory neurons whose dendrite cilia are ensheathed by AMsh glia and localizes to sensory dendrite cilia. We then searched for AMsh glia-enriched molecules involved in signal reception and found that loss-of-function mutants in *fig-1*, which encodes a transmembrane protein with extracellular thrombospondin domains, result in defects similar to those seen in *dgs-1* mutants. These findings suggest that glial FIG-1 senses the presence or activity of associated sensory dendrite cilia via neuronal DGS-1. Our studies reveal a previously uncharacterized signaling pathway by which neurons communicate their structure or functional state to surrounding glia, which respond by altering their secretion and transcription. We propose that similar signaling may occur at synapses, as glia-derived thrombospondin-domain proteins have been implicated in synapse assembly and function.

21 **Apoptotic trigger *egl-1* regulates mitochondria dynamics to promote exophers to maintain neuronal health and function** Zheng Wu, Jon PierceThe University of Texas at Austin

The Bcl-2 Homology 3-only (BH3) protein EGL-1 is a key initiator of apoptotic pathway in *Caenorhabditis elegans* and is conserved across metazoans. Previously, our lab discovered that *egl-1* is expressed throughout the life of the worm in the URX pair of sensory neurons without inducing apoptosis (Cohn et al. 2019). This expression depends cell-autonomously on neuronal activity and calcium. Similarly, orthologs of *egl-1* are expressed in neurons of the mammalian brain without causing cell death (Jiao et al. 2011). Non-apoptotic functions of *egl-1* in URX and its orthologs in mammalian neurons remain largely unclear.

In the process of studying URX, we noticed that it ejected exophers. Discovered in *C. elegans*, exophers are large membrane-surrounded vesicles that contain protein aggregates and organelles like mitochondria (Melentijevic et al.2017). Although they were first characterized in *C. elegans* touch neurons, more recently they were found in mammalian cardiomyocytes (Nicolás-Ávila et al. 2020).

We found that *egl-1* is required for exopher production cell-autonomously by promoting mitochondria fission in URX. The requisite role of *egl-1* in producing exophers extends to other neurons, including the touch neurons. We also found that *egl-1* was required for drug-induced production of exophers. We propose a model whereby a moderate level of *egl-1* expression acts as a stress response to promote the extrusion of damaged mitochondria and enable efficient mitochondria transport. Lack of *egl-1* can cause aggregation and mis-localization of the damaged mitochondria, which will impair the function of the neuron. Indeed, we find that although *egl-1* is dispensable for URX sensory reception (Cohn et al., 2019), it appears cell-autonomously required for URX to transmit downstream signaling to avoid high oxygen. Further study of the dependence of *egl-1* on exophergenesis may reveal more insight regarding the functions of BH3-only proteins in the mammalian brain health and function.

22 **Integration of spatially opposing cues by a single interneuron guides decision making in *C. elegans*** Asaf Gat[1], Vladyslava Pechuk[1], Sonu Peedikayil-Kurien[1], Gal Goldman[1], Jazz Lubliner[1], Shadi Karimi[2], Michael Krieg[2], Meital Oren-Suissa[11]Brain Sciences, Weizmann institute of science, [2]Neurophotonics and Mechanical Systems Biology, The Institute of Photonic Sciences

The capacity of animals to integrate and respond to multiple hazardous stimuli in the surroundings is crucial for their survival. In mammals, complex evaluations of the environment require large numbers and different subtypes of neurons. The nematode C. elegans avoid hazardous chemicals they encounter by reversing their direction of movement. How does the worms' compact nervous system processes the spatial information and directs the change of motion? We show here that a single interneuron,

AVA, receives glutamatergic excitatory signals from head sensory neurons and glutamatergic inhibitory signals from the tail sensory neurons. AVA integrates the spatially distinct and opposing cues, whose output instructs the animal's behavioral decision. We further find that the differential activation of AVA from the head and tail stems from distinct anatomical localization of inhibitory and excitatory glutamate-gated receptors along the AVA process, and from different threshold sensitivities of the sensory neurons to aversive stimuli. Our results thus uncover a cellular mechanism that mediates spatial computation of nociceptive cues for efficient decision-making in C. elegans.

23 **Resolving sensorimotor integration mechanisms of the RIA interneuron using a custom built microscope for calcium imaging in freely moving *C. elegans* (WormSpy)** Sebastian Wittekindt[1], Marie-Hélène Ouellette[2], Michael Hendricks[21]McGill, [2]Biology, McGill

C. elegans incorporate a variety of cues to effectively navigate their environment, avoid toxic threats, and find food. Assessing the way these signals are integrated in the animal's nervous system requires both information about the animal's pose and position within its environmental context, as well as a proxy for neural activity. To solve these challenges we designed a wide-field fluorescence tracking microscope that allows us to track single animals at sufficient magnification and resolution to synchronously capture the sub-cellular dynamics of neurons expressing genetically encoded calcium indicators, as well as behavioural dynamics under different environmental stimuli. We demonstrate WormSpy's advantages by applying it to the study of the RIA interneuron. RIA integrates head position and chemosensory signals via intracellular calcium signalling to mediate steering during chemotaxis, though the mechanisms for this are incompletely understood. Using WormSpy we demonstrate how spatiotemporal calcium signalling patterns give insight into the receptors and signalling motifs mediating chemotaxis.

24 **Coordination of head and body movement by electrically coupled interneurons AVG and RIF promotes roaming behavior** Tosif Ahamed[1], Wesley L Hung[1], Maggie Chang[2], Ying Wang[1], Ben Mulcahy[1], Yangning Lu[1], Jun Meng[1], Aravi Samuel[3], Mei Zhen[1,21]Lunenfeld-Tanenbaum Research Institute, [2]Molecular Genetics, University of Toronto, [3]Physics, Harvard University

C. elegans foraging behavior is defined by periods of continuous forward runs (roaming) and periods of increased turning and reversals to remain in an area rich in food (dwelling). Although, these states have been well characterized in terms of centroid position of worms, how they are controlled at the level of body wave dynamics is not well understood. We found that these states corresponded with distinct patterns of head-body coordination. Roaming involved increased coordination between head and body, whereas the opposite was true for dwelling.

To study this further, we developed an analysis pipeline that characterizes *C. elegans* body wave dynamics at different points along the body. With this pipeline, we measured head-body coordination in animals with mutations in *mod-1* with persistent roaming behavior, and in mutants of PDF pathway genes which have increased dwelling behavior, showing them to be distinct. Importantly, we identified a subcircuit composed of gap-junction connected interneurons AVG and RIF that coordinates the head and body during roaming and forward locomotion in general. We showed that stimulation of this subcircuit led to faster locomotion and increased neuronal activity in head motor neurons as well as AVB forward premotor interneurons and forward motor neurons. Consistent with their role in head-body coordination, AVG stimulation in AVB ablated animals silenced the body but led to increased head bending. While AVG stimulation with simultaneous silencing of head muscles led to body undulation without head movement. Genetic perturbations to the AVG/RIF circuit made the animals more likely to dwell, compromising their ability to efficiently explore food plates by roaming, further demonstrating their role in head-body coordination. Interestingly, in isolation from any synaptic inputs, AVG neuronal activity exhibited a slow rhythmic oscillation that is reminiscent of the timescale of the roaming/dwelling cycle.

Finally, we identified two genes that regulate roaming/dwelling behavior via this subcircuit. An RIF-specific protein, which inhibits roaming and a gene with AVG-specific expression, which promotes roaming.

In summary, our work reveals a novel component of the roaming/dwelling neural circuit that coordinates head-body movement. New genes that we identified may illuminate the mechanism for fine tuning of roaming/dwelling state switching.

25 **A command neuron in *C. elegans* orchestrates multiple motor outputs through parallel modes of transmission** Yung-Chi Huang[1], Jinyue Luo[1], Wenjia Huang[2], Casey Baker[1], Matthew Gomes[1], Alexandra Byrne[2], Steven Flavell[11]Massachusetts Institute of Technology, [2]University of Massachusetts Chan Medical School

Animals generate a wide range of motor outputs that are highly coordinated with one another, which allows them to execute purposeful behaviors. Individual neuron classes in the circuits that generate motor outputs have a remarkable capacity for flexibility, as they exhibit multiple axonal projections, transmitter systems, and modes of neural activity. How these multi-functional properties of neurons enable the generation of highly coordinated behaviors remains unknown. Here we show that the HSN command neuron in *C. elegans* evokes multiple motor programs over different timescales to enable a suite of behavioral changes

during egg-laying. Using HSN activity perturbations and in vivo calcium imaging, we show that HSN acutely increases egg-laying and locomotion while also biasing the animals towards low-speed dwelling behavior over longer timescales. The acute effects of HSN on egg-laying and high-speed locomotion are mediated by separate sets of HSN transmitters and different HSN axonal projections. The long-lasting effects on dwelling are mediated by HSN release of serotonin that is taken up and re-released by NSM, another serotonergic neuron class that directly evokes dwelling. Our results show how the multi-functional properties of a command neuron allow it to induce a coordinated suite of behaviors and also reveal for the first time that neurons can borrow serotonin from one another to control behavior.

26 **Using C. elegans to identify the GPCR targets of valproic acid, an anticonvulsant and mood-stabilizing drug** Lucero E Rogel-Hernandez[1], Emily K Fryer[1], Helena Casademunt[2], Aravinthan Samuel[2], Miriam B Goodman[11]Molecular and Cellular Physiology, Stanford University, [2]Physics, Center for Brain Science, Harvard University

Valproic acid (VPA) possesses both anticonvulsant and antimanic properties and has been widely prescribed to treat epilepsy, bipolar disorder, and other neuropsychiatric conditions for decades, but its molecular mode of action in the brain is not fully known. Furthermore, prenatal exposure to VPA is associated with birth defects, cognitive deficits, and an increased risk of autism. To gain insight into VPA's molecular targets in neurons, we exploited well-characterized chemosensation behaviors in C. elegans. In chemotaxis assays, we found that C. elegans is attracted to VPA and this behavior is missing in animals lacking the tax-4 ion channel and in the tax-4-expressing AWC chemosensory neurons. To test the idea that VPA directly activates the AWC neurons, we performed calcium imaging studies in a line expressing GCaMP6s in all chemosensory neurons [1] and found that VPA evoked calcium transients in AWC neurons. In a subset of recordings, calcium signaling in the AWB neurons was also affected. Given that chemosensory transduction typically begins with the activation of a G protein-coupled receptor (GPCR), we also determined how VPA attraction is affected in mutants with defects in Gα protein-encoding genes. In this way, we discovered that attraction to VPA depends on the egl-30 and gpa-10 Gα protein genes and is, therefore, likely to be mediated by one or more GPCR(s). To identify potential GPCR(s) targets for VPA, we mined two distinct single-cell RNAseq datasets (CeNGEN, Laurent) to build a list of candidate GPCRs. To date, we have tested 42 lines with defects in putative AWC-expressed GPCRs and identified a partial or complete loss of function in four GPCR genes. Future work is needed to determine if these VPA-worm GPCRs act alone or in concert and are directly modulated by VPA. Additionally, we view these GPCRs as novel entry points for understanding the mechanism of VPA action in humans.

27 **An inter-tissue feedback signal that couples muscle activity to glutamate receptor trafficking in distal upstream interneurons** Bethany Rennich, Molly Hodul, Peter JuoTufts Graduate School of Biomedical Sciences

It is becoming increasingly apparent that extracellular signals can modulate synaptic strength by controlling the number of AMPA-type glutamate receptors (GluRs) in the postsynaptic membrane. However, we are only beginning to understand the mechanisms by which secreted factors influence glutamatergic synaptic strength, especially those mediated by signals that act at a distance between different tissues. *C. elegans* express many putative extrasynaptic secreted signaling molecules, including neuromodulators and over 200 neuropeptides (many with unknown function), that could potentially regulate synaptic and circuit function. Here, we identify an inter-tissue signal that couples changes in muscle activity with GLR-1/GluR surface abundance in distal upstream interneurons. Mutants lacking the neuromuscular junction (NMJ) acetylcholine receptor (AChR) subunits *unc-29* or *unc-38* exhibit a compensatory increase in surface levels of GLR-1 in the locomotion command interneuron AVA. This increase in surface GLR-1 could be rescued by expressing wild type cDNA of *unc-29* specifically in the body wall muscle, revealing a feedback pathway that couples NMJ signaling with GLR-1 trafficking in AVA. Chronic loss of muscle contraction in *unc-54*/muscle myosin mutants also results in increased surface GLR-1 levels in AVA, suggesting that lack of muscle contraction is sufficient to trigger the feedback pathway. Acute muscle inactivation, induced with temperature-sensitive alleles of *unc-54*/myosin or *twk-18*/potassium channels, was sufficient to trigger the feedback pathway in larval L4 animals, suggesting that the change in surface GLR-1 levels can be engaged on a relatively short timescale and cannot be attributed to a developmental defect. Loss of function mutations in *unc-31*/CAPS, which mediates the release of neuropeptide-containing dense-core vesicles, blocks the feedback pathway triggered by either *unc-29*/AChR or *unc-54*/myosin mutants. Expression of *unc-31* in muscle partially rescues this defect. A focused screen to identify muscle-expressed neuropeptides that mediate this feedback pathway is ongoing. Together, our results identify a novel inter-tissue signal that couples muscle activity with surface GluR levels in upstream interneurons. We propose that this compensatory feedback signal adjusts motor circuit excitability in response to changes in muscle contraction and may be engaged under conditions of declining muscle function.

28 **Conflict during learning reconfigures the neural representation of positive valence and approach behaviour** Laura Molina-Garcia[1], Susana Colinas-Fischer[2], Sergio Benavides-Laconcha[1], Lucy Lin[1], Emma Clark[2], Neythen J Treloar[2], Blanca Garcia-Minaur-Ortiz[1], Chris P Barnes[2], Arantza Barrios[21]University College London, [2]Cell and Developmental Biology, University College London

A central goal in neuroscience is to understand how neural circuits integrate conflicting (rewarding and punishing) experiences during learning which need to be behaviourally resolved. To shed light into the molecular and cellular mechanisms underlying this process we have dissected the neuronal circuit underlying sexual conditioning in *C. elegans*. *C. elegans* learn to avoid an innately attractive odour, when it is paired with starvation. In males, this aversive learning can be overridden by sexual conditioning. Here, the odour is conditioned with both a punishment (starvation) and a reward (mates) resulting in odour approach.

We found that rewarding and punishing experiences are both encoded by the neuropeptide PDF-1, being released from and acting on different neurons. By using a Cre-Lox intersectional strategy, we identified the interneurons RIM RIA and AIY as target cells of PDF-1 neuromodulation, and the MCM and AVB neurons as the relevant sources of PDF-1 neuromodulation during sexual conditioning. Furthermore, we found that the AVBs are activated by mate experience during memory acquisition.

Because in sexual conditioning, mate experience overrides aversive learning resulting in odour approach instead of avoidance, we proposed two alternative models of how conflicting cues might be integrated. In model 1, mate presence inhibits the formation of the aversive memory. In model 2, the aversive memory still forms but the presence of mates creates a parallel memory which results in approach. To understand how odour information is represented in the circuit after conditioning and PDF-1 neuromodulation we measured neuronal activity within the circuit. We found PDF-1-dependent activity changes in AIY specifically in sexually conditioned animals. Moreover, we found traces of the aversive memory in sexually conditioned worms, demonstrating that each experience (rewarding and aversive) creates a separate, parallel memory in the circuit for odour processing, supporting model 2. This results in the sensorimotor representation of odour being different in naïve and sexually conditioned animals despite both displaying approach. To relate activity to behaviour we built a computational model to show that the increased AIY activity displayed by sexually conditioned animals could lead to odour approach. Our results reveal that the positive valence of a stimulus is flexibly represented within the circuit according to the experiences and predictions associated with the stimulus.

29 **Distributed encoding of motor commands mediates response to environmental confinement and escape from predators** Itamar Lev, Stephanie Josephine Eder, Manuel ZimmerDepartment of Neuroscience and Developmental Biology, University of Vienna

Natural behavior requires flexible adjustments, for example when navigating complex environments or escaping predators. Previously, whole-brain recordings in *C. elegans* uncovered that a large proportion of neuronal activity relates to motor commands. However, the role of these distributed motor commands remains elusive. Here, we show that movement in hard-to-move environments provokes longer reversals that coincide with persistent activity of reversal interneurons, indicating an increased effort to execute the behavior. Utilizing whole-brain recordings, we find that the distributed reversal states include the sensory neurons URYs and OLQs. Manipulating these neurons, we show that these sensory neurons stabilize the prolonged reversal state and behavior. Moreover, we find that the OLQs are *a priori* activated during forward movement in hard-to-move environments, enhancing reversal duration once initiated. By conducting a genetic screen for regulators of reversal duration, we uncovered molecular components acting in URY, OLQ, and motor neurons underlying positive and negative feedback on reversal duration. For example, we find that URY and OLQ neurons elongate reversal duration via glutamate signaling. Next, we examined the animal's interaction with a natural predator, the nematode-trapping fungus *A. oligospora*. We show that prolonged reversals and the above circuitry play a role in escaping the fungal traps. In conclusion, our data supports a model where distributed motor commands involving sensory neurons enable adaptive motor responses that are vital in natural environments.

30 **A Data Modelling Framework for Functional Annotation of the *Caenorhabditis elegans* Connectome** Sharan J Prakash[1], Kimberly Van Auken[1], David P Hill[2], Paul W Sternberg[1]1Biology & Biological Engineering, California Institute of Technology, [2]The Jackson Laboratory

The anatomically compact nervous system of *C. elegans* offers an opportunity to understand the structure-function relationship of the entire brain of an animal. One approach towards this understanding is to compile the large volume of experimental results, generated by studies of individual neural circuits, within a connectome context. In this way, a brain-wide map of relationships between environmental inputs, gene functions, neural circuits and behavior could be visualized. This can be described as functional annotation of the connectome. An important problem in this endeavour is to ensure that the underlying data are accurately represented in a machine-readable format. In this work, we describe how a data modelling framework developed by the Gene Ontology Consortium (GOC), Gene Ontology-Causal Activity Modelling (GO-CAM) can be adapted to provide a rigorous basis for connectome annotation. GO-CAM extends atomic information about individual genes to create knowledge graphs representing biological processes using a defined set of 'relations' (or edges) to connect genes, GO Molecular Function, GO Biological Process, and GO Cellular Component terms (nodes), and thus generates networks describing the causal relationships between gene activities in their appropriate context. We explored whether GO-CAM can be adapted to represent causal relationships between environmental inputs, gene functions, neural circuits, and behavior in *C. elegans*. We found that, given modifications, a wide variety of experimental results on the egg-laying and carbon dioxide (CO_2) avoidance circuits could be

faithfully represented with GO-CAM. Thus, we demonstrate how to create machine-readable semantic models of neural circuits, integrated with knowledge of *C. elegans* gene function. Through this empirical exercise, we were able to generate generic data models or 'curation templates' for several important categories of experimental results. We term these models *Ce*N-CAM (*C. elegans* Neural Circuit-Causal Activity Modelling). The generic models will enable development of an intuitive user interface for experimental scientists and biocurators to contribute to connectome annotation, without prerequisite knowledge of the *Ce*N-CAM data model. We also discuss how connectome annotation can be used to accelerate research in *C. elegans* systems neuroscience. We hope this work will motivate dedication of resources and effort in synthesising knowledge of *C. elegans* neurobiology.

31 **A genetic toolkit for measuring functional connectome in *Caenorhabditis elegans*** Anuj Sharma[1], Francesco Randi[1], Sandeep Kumar[2], Sophie Dvali[1], Andrew Leifer[31]Physics, Princeton University, [2]Princeton Neuroscience Institute, Princeton University, [3]Physics & Princeton Neuroscience Institute, Princeton University

We present a suite of transgenic lines to optically measure direct neuronal connections at brain scale and cellular resolution in the nervous system of the nematode *C. elegans*. Using these strains, we have identified peptidergic signaling contribute significantly to neural dynamics in the animal's head[1].

We generated transgenic animals that express the calcium indicator GCaMP6s in each neuron and a purple-shifted light-sensitive actuator, the gustatory receptor homolog system GUR-3+PRDX-2, in each neuron [2]. This combination of indicator and activator allows for 2-photon targeted optogenetic stimulation during simultaneous 1-photon calcium imaging with minimal optical cross-talk. To achieve high expression levels while avoiding toxicity, we used the QF-GR drug-inducible system to turn on gene expression only in adulthood [3]. These optogenetic tools were expressed in a NeuroPAL background to allow each neuron to be uniquely identified [4].

Neuropeptides and neuropeptide receptors are broadly expressed across the *C. elegans* nervous system, but it remains unclear what role they play, the timescales on which neuropeptide signaling acts, and which putative connections are functional. To map out peptidergic signaling dependent calcium transients, we stimulated individual neuron while measuring the network's calcium response at cellular resolution across the brain. We compared responses in WT-background and *unc-31(wtf509)* CRISPR Cas9 KO animals that had defects in dense core vesicle (DCV) signaling. We identified ~75 neuron pairs for which neuron stimulation evoked a DCV dependent calcium response in a downstream neuron. We used recently accessible gene expression data and de-orphanization studies to identify peptides and receptor pairing expressed in many of these neuron pairs that are consistent with peptidergic signaling. In addition, new lines with *unc-13 (s69)* & unc-13 *(e51)* as well as *unc-9 (fc16);unc-7(e5)* mutant back grounds by out crossing and *inx-1(wtf511)* & *inx-7 (wtf514)* by CRISPR Cas9 KO are created for further investigations into neurotransmitter and gap-junction mediated signaling.

This transgenic toolkit will be a resource for large scale investigations of functional connectivity in the brain.

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32 **Neural signal propagation atlas of *C. elegans* reveals that extrasynaptic signaling contributes to brain dynamics**

Francesco Randi[1], Anuj K Sharma[1], Sophie Dvali[1], Andrew M Leifer[1,21]Physics, Princeton University, [2]Neuroscience, Princeton University

A central question in neuroscience is how properties of a network of neurons relate to the network's function. One approach to answering this question can be found in the anatomical connectome. To investigate this we systematically measure signal propagation in 9,692 pairs of neurons across the head of the nematode *C. elegans* via direct optogenetic activation of individual neurons and simultaneous calcium imaging of the whole brain to create a functional atlas. We find that our functional atlas differs from predictions made based on the anatomical connectome. In addition to the anatomical connectome, recent studies (Ripoll-Sánchez et. al 2022) show that extrasynaptic, "wireless", signaling via neuropeptides plays an important role in the nervous system. Using mutants we show that extrasynaptic signaling, which is not visible in anatomy, contributes to the difference between anatomy and functional connectivity. We identify many instances of dense-core-vesicle dependent signaling

on seconds-or-less timescales that evoke acute calcium transients— often where no direct wired connection exists but where relevant neuropeptides and receptors are expressed. This suggests that neuropeptides that are released extrasynaptically via dense-core-vesicles can perform a similar role to that of classical synaptically released neurotransmitters. Additionally, we compare both anatomy and our signal propagation atlas to spontaneous activity and found that the neural dynamics of spontaneous activity were better predicted by signal propagation.

33 **Influenceability and predictability of C. elegans action selection through closed-loop interrogation** Raymond L Dunn[1], Julia Miller[2], Jackson Borchardt[1], Noelle D L'Etoile[2], Saul S Kato[11]Neurology, University of California, San Francisco, [2]Cell and Tissue Biology, University of California, San Francisco

The C. elegans brain is an active nonlinear control system, which combines time-varying sensory input from the environment with evolving internal state to produce effective continuous behavioral output. Whole-brain calcium imaging has revealed that the worm brain cycles through broadly distributed, coordinated patterns of activity which correspond to major motor behavioral states, such as forward and reverse crawling and turning. However, whole-brain dynamics studies have been largely correlative. Causal aspects of neural network activity, such as how distinct sequences of activity are produced and engaged to transition the worm between behaviors, remain unknown.

To evaluate the neural basis of behavioral control, we turn to single-cell optogenetics during whole-brain imaging to study the influence of precisely-timed perturbations on future neural dynamics. We ask whether behavioral state sequences are determined by the time evolution of a low-dimensional network state; termed the stable manifold hypothesis, which predicts that certain perturbations of the network should, at critical times of global brain state cycle, influence future behavioral state.

We developed a closed-loop system to address this question, combining approaches of targeted expression of a red-shifted depolarizing opsin, patterned illumination with a digital micro-mirror device, and real-time analysis of neural activity to time optogenetic activation of single neurons. Furthermore we conduct our experiments in NeuroPAL, enabling identification of neurons by position and multi-channel fluorophore expression.

We probed the influenceability of brain state trajectory by brain state-triggered optogenetic activation of several neurons including OLQV, OLQD, SMDV, SMDD, ASH, ASI, RIA, RMEV, RMED, RMDV, and RMDD while simultaneously recording whole-brain activity. We demonstrate that ASH activation elicits the neural correlate of reversal, measured by depolarization of the AVA neuron. However, surprisingly we find that SMDV and SMDD activation, as well as sub-cellular activation of distinct regions of RIA, sometimes halts ongoing reversal commands. By machine learning analysis of network activity, we find that the stochasticity of response disappears and the low-dimensional brain-wide neural state at the time of stimulation determines the response of the animal, substantiating the stable manifold hypothesis.

We then constructed dynamical state space models of network activity, trained on our experimental data, and found that these models better predict the response to perturbations versus static models, lending further support to the stable manifold hypothesis and the deterministic dynamical systems view of C. elegans neural control of behavior.

34 **Towards routine reconstruction of *C. elegans* connectomes, cell states, and cell types, through optimized expansion microscopy** Yangning Lu[1,2], Chi Zhang[1,2], Madison A Sneve[1,2], Tay Won Shin[1,2], Chih-Chieh Yu[1,2], Bobae An[1,2], Abigail M Mauermann[1,2], Seung Hyeon Shim[1,2], Edward S Boyden[1,21]Massachusetts Institute of Technology, [2]Howard Hughes Medical Institute

Connectomes, comprehensive diagrams of the entire nervous system's wiring, offer the chance to link the intricate circuitry of the brain, to emergent dynamics and subsequent behavior. The current method for mapping connectomes at the nanoscopic level, serial section electron microscopy (ssEM), has remarkable spatial resolution but faces challenges in molecular identification, which is crucial to interpreting connectomes in terms of physiological properties. Additionally, ssEM requires expensive hardware and specialized expertise. A simple and inexpensive connectomics method would allow routine analysis of cell connectivity, cell types, and cell states in neuroscience research.

By optimizing and applying our recent invention of expansion microscopy (ExM), which through physical magnification of biological specimens enables nanoimaging to be performed on ordinary microscopes (Science, 347(6221), 543-548), such inexpensive and simple molecularly-annotated connectomics may soon be possible. The nematode worm, *C. elegans*, remains the only species with a completely mapped connectome (by ssEM). We aimed to utilize ExM to reconstruct the neuronal connections of *C. elegans* with molecular identity. However, *C. elegans* is covered by a tough cuticle that hinders expansion. To overcome this challenge, we developed a method of embedding worms in a hydrogel equipped with proteases, which removes the cuticle without affecting internal molecules. This allowed us to isotropically expand the worms to high expansion factors, while retaining molecular information.

By expansion and immunostaining, both exogenously expressed and endogenous proteins, including synaptic proteins, could be revealed with nanoscale resolution. To trace the neurons, we employed cell-type-specific fluorescent proteins to differentiate and encode neighboring neurites. The color code physically segments the neurons, potentially facilitating tracing. To ensure continuity of tracing signals, we immunostained tubulins that are highly expressed by neurons. Through these efforts, we are achieving delineation of neurites and annotation of synapses by expansion microscopy in a high throughput fashion. Our method provides a path towards reconstructing complete connectomes in everyday neuroscience, unveiling the wiring, molecular composition, cell types, and states, after functional experiments are conducted.

35 **The cortical microtubule regulator EFA-6 forms spatially restricted condensates dependent on its intrinsically disordered region and interactions with tubulins** ANJALI SANDHU, Xiaohui Lyu, Xinghaoyun Wan, Xuefeng Meng, Ngang Heok Tang, Andrew Chisholm, Yishi JinDepartment of Neurobiology, University of California

Microtubules (MTs) are dynamic components of the cytoskeleton and play essential roles in morphogenesis and maintenance of tissue and cell integrity. Regulation of MTs at the cellular cortex ensures distinct cell shapes and connections with extracellular matrix and other cells. The conserved protein EFA-6 functions in multiple cellular processes from embryonic cell division to neuronal pruning and regeneration through destabilizing cortical MTs via an 18 aa MT elimination domain (MTED) (O'Rourke et al., Nat Cell Biol, 2010; Chen et al., Elife, 2015). To identify regulators of EFA-6 expression, we first characterized EFA-6 endogenous expression using GFP knock-in. As expected, GFP::EFA-6 localizes uniformly at the cell cortex in multiple cell types throughout larvae and adults. Interestingly, in the adult epidermis EFA-6 forms condensates at the dorsoventral boundaries of lateral epidermal ridges where longitudinal and circumferential MTs intersect. We show that formation of these EFA-6 condensates is dependent on its intrinsically disordered N-terminal region. In a visual genetic screen for mutants with altered GFP::EFA-6 expression, we identified a gain-of-function mutation in the alpha-tubulin *tba-1* that induces ectopic EFA-6 condensates in multiple tissues. The ectopic EFA-6 condensates in TBA-1(gf) require the MTED domain of EFA-6. In vivo imaging analysis of GFP::TBA-1 shows that the mutant TBA-1(gf) is compromised in its incorporation into epidermal MTs. Pharmacological treatment suggests that TBA-1(gf) may specifically alter tubulin heterodimer formation. *tba-1*(gf) animals exhibit temperature-sensitive late embryonic lethality, with majority of embryos arrested around morphogenesis. Mutant EFA-6 with reduced ability to form condensates can partially suppress this embryonic lethality. While published work indicates EFA-6 regulates MT dynamics, our data reveal a feedback regulation between tubulins and EFA-6 such that the regulation of the cortical MT cytoskeleton by EFA-6 may be modulated by its ability to form local condensates via tubulins.

36 **A new methyl-mark regulating neuronal function "written" on microtubules by the histone methyltransferase NSD3/MES-4** Edward W Pietryk[1], Rahul Jangid[1], Durga N Tripathi[1], In Young Park[1], Anish Thachangattuthodi[1], Neetu LNU[1], Xiaoli Wang[1], Sung Yun Jung[1], Ruhee Dere[1], B.V.V Prasad[1], David J Reiner[2], Rachel Arey[1], Cheryl L Walker[11]Baylor College of Medicine, [2]Texas A&M Health Institute of Biosciences and Technology

Analogous to the "Histone Code" for how histone post-translational modifications (PTMs) determine chromatin function, conformation, and integrity, a "Tubulin Code" is being developed to understand how various PTMs determine microtubule structure and function. Lysine methylation, one of the most prominent PTMs of the "Histone Code" was recently shown to also contribute to the "Tubulin Code", and serve important functions on microtubules. Interestingly, the same enzymes responsible for methyl marks on histones have been shown to be responsible for "reading, writing and erasing" methyl marks on microtubules. However, genotype-phenotype correlation studies to determine function of tubulin PTMs are hampered by the presence of multiple tubulin genes and isoforms expressed in virtually all mammalian cells. In *C. elegans* however, the six touch receptor neurons (TRNs) are highly enriched for a single isotype of α-tubulin: *mec-12*. Thus, studies of α-tubulin PTMs in TRNs can overcome the complexity and redundancy of multiple α-tubulin isotypes present in mammalian cells. We recently identified a new methyltransferase that "writes" di-methyl marks on both histones and α-tubulin: NSD3. To investigate the functional role of di-methyl PTMs "written" by NSD3 on microtubules *in vivo*, we generated a CRISPR knock-in *C. elegans* line with an endogenously tagged MEC-12::mKate2 reporter. In this MEC-12::mKate2 line, we generated mutants lacking the sole *C. elegans* NSD methyltransferase orthologue, *mes-4,* and a MEC-12::mKate2 line where the lysine residues methylated by NSD3 were mutated to alanine (K-to-A mutations). Using these methyl-deficient mutant lines, we found α-tubulin methylation plays an important role in TRN function, with loss of either the "writer" *mes-4/NSD* or its lysine targets in the K-to-A *mec-12/α-tubulin* mutant displaying defects in touch response. Along with behavioral deficits, methyl-deficient mutants showed defects in TRN morphology and abnormal microtubule dynamics when compared to wild-type animals. Together with cellular and biochemical data obtained in mammalian cells, these *C. elegans* studies provide functional data identifying NSD3 as a new di-methyltransferase for α-tubulin, and an exciting new role for microtubule methylation in neuronal morphology and function *in vivo*.

37 **LIN-5 (NuMA) regulates cytokinesis furrow formation independent of its role in spindle positioning** Kuheli Adhikary[1], Sukriti Kapoor[2], Sachin Kotak[21]Microbiology and cell biology, Indian Institute of Science, [2]Microbiology and Cell Biology, Indian Institute of Science

Proper assembly of the cleavage furrow is a key to error-free cell division. In animal cells, midzone localized RhoGEF ECT-2 is essential for appropriate RhoA enrichment and, thus, proper furrow formation. How ECT-2 localization/activity is spatiotemporally regulated to control cleavage furrow formation remains incompletely understood. In *the Caenorhabditis elegans* zygote, the spindle midzone localized centralspindlin complex (CYK-4/ZEN-4) promotes cleavage furrow formation by activating RhoGEF ECT-2 at the equatorial membrane. Notably, in the absence of centralspindlin, the timing of furrow formation is intact. However, the cytokinetic furrow fails to ingress fully, leading to cytokinesis failure. LIN-5 (NuMA in humans) is an evolutionarily conserved coiled-coil protein essential for spindle positioning and is critical for timely furrow formation. However, how LIN-5 regulates cytokinetic furrow formation remained elusive. In this work, we discovered that LIN-5 regulates timely furrow formation independent of its function in spindle positioning in the *C. elegans* zygote. This finding was further strengthened in AB blastomere that does not rely on the LIN-5-based pathway for spindle positioning. Interestingly, our data reveal that LIN-5 acts redundantly with centralspindlin in controlling the stability of the cytokinetic furrow. Furthermore, ectopic targeting of centralspindlin at the equatorial membrane rescues the cytokinetic furrow delay seen upon LIN-5 depletion, suggesting that LIN-5 depletion delays furrow by affecting the localization/activity of CYK-4/MKLP-1/ECT-2 complex. Overall, our data indicate that the polar cortical localization of the LIN-5-based complex restricts the equatorial cortical enrichment of CYK-4/MKLP-1/ECT-2 to regulate RhoA for timely cleavage furrow formation.

38 **Force-generation in the cytokinetic ring aligns the AB cell division with egg shell geometry** Teije Middelkoop[1], Jonas Neipel[2], Caitlin Cornell[3], Lokesh Pimpale[4], Frank Jülicher[2], Stephan Grill[41]Institute of Molecular Genetics of the Czech Academy of Sciences, [2]Max-Planck-Institute for the Physics of Complex Systems, [3]University of California, Berkeley, [4]Max Planck Institute of Molecular Cell Biology and Genetics

Pioneering work performed more than a century ago showed that cells tend to divide along their long axis. Several underlying mechanisms have been put forward that either involve force generation in the mitotic spindle apparatus, in the actomyosin cortex, or in both. However, whether and how both types of cytoskeletal forces are coordinated remains unclear. Here we report a novel mechanism by which Myosin/NMII-driven contractility in the cytokinetic ring aligns the cell division axis of the C. elegans AB blastomere with its long axis. This alignment involves a full-body rotation of the entire 2-cell embryo within its stationary egg shell. By combining the strength of C. elegans genetics with 3D time-lapse microscopy, quantitative image analysis and physical modeling, we show that 1) rotation coincides with actomyosin ring formation, 2) is counteracted by dynein-mediated cortical pulling on the mitotic spindle and 3) is the result of Myosin/NMII-driven ring ingression. This mechanism can explain how early embryonic cells undergoing rapid rounds of division align their division axis with egg shell geometry.

39 **Microtubule force generators govern spindle orientation in mitotic *C. elegans* germ cells** Reda M. Zellag[1,2], Vincent Poupart[1], Abigail R. Gerhold[2], Jean-Claude Labbé[11]IRIC, Université de Montréal, [2]Department of Biology, McGill University

The three-dimensional architecture of a tissue is intimately linked to its function. Tissue architecture is influenced by the orientation of the mitotic spindle, which sets the cell division plane in response to cues such as cell adhesion. *C. elegans* germ cells are organized in a circumferential monolayer around a shared core of common cytoplasm, known as the rachis, to which each cell is open via a single cytoplasmic bridge. Mitotic germ cells were previously reported to lack canonical cell-cell junctions and to divide in a random orientation, and so how this tissue architecture arises and is maintained during animal development is not clear. We have developed 3D image segmentation and analysis tools to measure spindle orientation in live-imaged germ cells relative to cell axes and tissue landmarks. We find that spindle orientation is strongly biased, confining germ cell division within a plane parallel to the rachis surface. Within this plane, germ cells preferentially divide along the axis of gonad elongation. Depleting the microtubule force generator dynein by auxin-mediated degradation, or perturbing the activity of the conserved dynein regulator LIN-5 (NuMA), reduced the spindle orientation bias relative to the rachis surface. Analysis of LIN-5 localization in germ cells revealed that while it is enriched at the basolateral membrane during interphase, its cortical levels decrease markedly during mitosis, with the remaining LIN-5 becoming enriched on lateral cell cortices adjacent to the spindle poles. We propose that the dynamic localization of LIN-5 regulates the cortical activity of microtubule force generators to orient the mitotic spindle parallel to the surface of the rachis, thus maintaining tissue architecture during germ cell division.

40 **Phosphorylation of ZYG-1 at Multi-Sites Regulates ZYG-1 Stability and Centrosome Number** Mi Hye Song, Jeffrey C Medley, Nahyun Yim, Joseph DiPanniBiological Sciences, Oakland University

During cell division, equal distribution of chromosomes into each daughter cell is essential to maintain genomic integrity. As microtubule-organizing centers, two centrosomes establish the mitotic bipolar spindles and promote accurate segregation of genomic content. To maintain proper centrosome number, centrosome assembly must be tightly controlled by duplicating only once per cell cycle. Protein phosphorylation has emerged as a key mechanism that influences the activity of centrosome proteins. In *Caenorhabditis elegans*, the Plk4-related kinase ZYG-1 is required for centrosome duplication. The protein kinase CK2 has been shown to negatively regulate centrosomal ZYG-1 levels. In this study, we investigated the functional impact of ZYG-

1 phosphorylation, potentially by CK2, in regulating centrosome duplication. We show that CK2 directly phosphorylates kinase-dead ZYG-1 *in vitro* and CK2/KIN-3 physically interacts with ZYG-1 in embryonic lysates. Using *in silico* tools, we identified several ZYG-1 residues conforming to consensus CK2 target sites, and focused on four serine sites within the ZYG-1-Linker1 domain that is critical for ZYG-1 loading to centrosomes and the ZYG-1-SAS-6 binding. To test the *in vivo* role of phosphorylation of ZYG-1*,* we generated the *C. elegans* strains carrying mutations at the four serine residues replaced with alanine (S-to-A: Non-Phosphorylatable; NP) or aspartic acid (S-to-D: Phospho-Mimetic; PM) at the endogenous loci using CRISPR/Cas9 editing. We show that the NP-ZYG-1 mutations lead to elevated levels of cellular and centrosomal ZYG-1, and restore bipolar spindles and embryonic viability to hypomorphic *zyg-1* mutants, suggesting that the NP-ZYG-1 mutation stabilizes ZYG-1. As expected for hyper-stabilized ZYG-1, similar to overexpression of ZYG-1, extra centrosomes are often observed in the NP-ZYG-1 mutant embryos. By contrast, the PM-ZYG-1 mutations lead to reduced ZYG-1 levels and aggravate *zyg-1* mutant phenotypes. Finally, we show that inhibition of the 26S proteasome partially blocks degradation of the unstable PM-ZYG-1 form, while the stable NP-ZYG-1 form becomes partially resistant to proteasomal degradation. Together, our data support a model where phosphorylation of ZYG-1 at multi-sites regulates ZYG-1 stability via proteasomal degradation. Therefore, site-specific phosphorylation of ZYG-1 provides an additional mechanism to fine-tune ZYG-1 levels during cell cycle progression, leading to one and only one centrosome duplication in early *C. elegans* embryos.

41 **PCMD-1 bridges the centrioles and the PCM scaffold** Tamara Mikeladze-Dvali[1], Lisa Stenzel[1], Alina Schreiner[1], Elisa Zuccoli[1], Sim Üstüner[1], Judith Mehler[1], Esther Zanin[21]Biology, Biozentrum der LMU, [2]Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg

Centrosomes are the main microtubule organizing centers of animal cells. They comprise a pair of barrel shaped centrioles surrounded by the pericentriolar material (PCM). Microtubule nucleation at the spindle poles is mediated by PCM components, which assembles around a pair of centrioles. Although centrioles were demonstrated to be essential for the recruitment of the PCM scaffold (Cabral et al., 2019), proteins which anchor the PCM to the centrioles have not been described in *C. elegans*.

Recently we identified a new centrosomal protein Pericentriolar Matrix Deficient-1 (PCMD-1). In one-cell embryos with compromised PCMD-1 function centrioles fail to assemble the non-mitotic PCM core. At mitotic entry PCM scaffold forms to a certain degree, but it often fails to localize to centrioles (Erpf et al., 2019). Here we present evidence that PCMD-1 plays a role in tethering the PCM to the centrioles.

We demonstrate that the centrosomal recruitment of PCMD-1 is dependent on the outer centriolar protein SAS-7. We show that PCMD-1 is interacting with the PCM scaffold protein SPD-5, the mitotic kinase PLK-1 and the centriolar protein SAS-4. Using an ectopic translocation assay, we show that PCMD-1 can selectively recruit downstream PCM scaffold components to an ectopic location in the cell, indicating that PCMD-1 is able to anchor the PCM scaffold proteins at the centrioles. Furthermore, our structure-function analysis revealed that the most C-terminal part of PCMD-1 is sufficient for the interaction with SAS-4 and to target PCMD-1 to the centrosome. Together, we propose that in *C. elegans* PCMD-1 is an essential, functional bridge between the centrioles and the PCM.

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42 **The interkinesis envelope is a novel organelle that covers chromosomes in *C. elegans* oocytes** Layla El Mossadeq[1], Rémi Le Borgne[1], Kimberley Laband[1], Hélène Geoffroy[1], Peter Askjaer[2], Jean-Marc Verbavatz[1], Julien Dumont[11]Université de Paris Cité, CNRS, Institut Jacques Monod, F-75013 Paris, France, [2]Andalusian Centre for Developmental Biology, Consejo Superior de Investigaciones Científicas (CSIC), Universidad Pablo de Olavide, Sevilla, Spain

Cell division is essential for the development, homeostasis and reproduction of organisms. In eukaryotes, exit from interphase and entry into cell division are marked by the nuclear envelope breakdown and the condensation of chromosomes. In contrast, at the end of cell division, when the cell enters back in interphase, the nuclear envelope reforms and surrounds the decondensing chromosomes. The reproduction of organisms requires haploid gametes (oocytes and spermatozoa) generated during meiosis. Meiosis corresponds to two successive rounds of chromosome segregation separated by a short phase, called interkinesis. During interkinesis in oocytes, chromosomes remain condensed. It has thus been proposed that interkinesis does not correspond to a proper interphase, and that the nuclear envelope does not reform at this stage. However, our work in *Caenorhabditis elegans* oocytes led us to identify a double membrane surrounding the surface of the condensed chromosomes in interkinesis.

However, by combining light and electron microscopy in *Caenorhabditis elegans* oocytes, we surprisingly found that a double membrane covers the surface of the condensed chromosomes during interkinesis. The interkinesis envelop is assembled transiently between anaphase I onset and the entry into meiosis II. It is comprised of the lamina protein LMN-1 and inner nuclear membrane proteins LEM-2, SUN-1 and EMR-1, but lacks outer membrane proteins ZYG-12 and SP-12, which is consistent with the observed discontinuity between the interkinesis envelope and the endoplasmic reticulum. Surprisingly, although nuclear pores are absent, some nucleoporins localized at the interkinesis envelope. Functional analysis revealed that formation of the interkinesis envelope depends on the nucleoporin MEL-28^{ELYS}, which recruits membranes on the segregating chromosomes, and on the chromatin binding protein BAF-1^{BAF}, which ensures interkinesis envelope integrity. Our results also demonstrate that the interkinesis envelope is never completely sealed, and accordingly does not involve the function of the ESCRTIII complex, which is normally required for the sealing of interphase nuclear envelopes.

Finally, experimental perturbation of the interkinesis envelope led to faster meiotic chromosome segregation, suggesting that this newly identified structure participates in correct chromosome segregation in the *C. elegans* oocytes.

43 **Sexually dimorphic regulation of meiotic recombination by the *C. elegans* synaptonemal complex proteins** Cori Cahoon, Colette Richter, Amelia Dayton, Diana LibudaUniversity of Oregon

In sexually reproducing organisms, germ cells faithfully transmit the genome to the next generation by forming haploid gametes, such as eggs and sperm. Although most meiotic proteins are conserved between eggs and sperm, many aspects of meiosis are sexually dimorphic. The mechanisms regulating recombination display sex-specific differences in multiple organisms such that the same proteins in each sex are utilized in different ways to produce sexually dimorphic outcomes. The synaptonemal complex (SC), a large ladder-like structure that forms between homologous chromosomes, is essential for regulating meiotic chromosome organization and promoting recombination. While both sexes use the same SC structure in their germ cells, how the SC accommodates these sexually dimorphic aspects of meiosis while regulating recombination remains unknown. Using novel tools we developed for sex comparative imaging studies in *Caenorhabditis elegans*, we identified sex-specific roles for two SC central region proteins, SYP-2 and SYP-3, in regulation of meiotic recombination. With quantitative live and fixed imaging, we find that SC composition is regulated by sex-specific mechanisms throughout meiotic prophase I. During prophase I, both oocytes and spermatocytes differentially regulate the stability of SYP-2 and SYP-3 within an assembled SC, with increased SYP-2 dynamics in spermatocytes and increased SYP-3 dynamics in oocytes. Further, we uncover that the relative amount of SYP-2 and SYP-3 within the SC is independently regulated in both a sex-specific and recombination-dependent manner. In the absence of recombination, oocyte SYP-2 and SYP-3 levels increase in abundance, while spermatocyte SYP-2 and SYP-3 levels remain relatively unchanged indicating different dosage-dependent mechanisms regulating each SC central region protein during recombination. Using cytological markers of specific recombination stages and genetic recombination assays, we find that SYP-2 specifically regulates the early steps of recombination in both sexes, while SYP-3 controls the timing and positioning of crossover recombination events across the genomic landscape differently in each sex. Taken together, we demonstrate that the individual components of the SC have specific sex-specific functions in recombination and are not uniformly regulated. These sexual dimorphic features of the SC provide insights into how spermatogenesis and oogenesis adapted similar chromosome structures to differentially regulate and execute recombination.

44 **The C-terminus of SYP-4 regulates crossover formation in *C. elegans* meiosis** Ana Rita Rodrigues Neves, Simone KöhlerEMBL

During meiosis, crossover formation between homologous chromosomes establishes a physical link between the homologs. This link is crucial for meiosis as it guarantees that the homologs will be properly oriented and segregated correctly in the first meiotic division. Crossover formation is tightly regulated to ensure that at least one crossover per pair of homologs is formed (crossover assurance). Additionally, crossovers are not distributed randomly along the chromosomes but are spaced far apart (crossover interference). However, how crossover formation is regulated and how the signals for crossover assurance and interference are propagated is not fully understood. Recent observations in *Caenorhabditis elegans* and plants led to the proposal of a model based on the coarsening of crossover promoting molecules such as ZHP-3 along the synaptonemal complex, a proteinaceous structure assembled between the homologous chromosomes. The accumulation of ZHP-3 at the crossover site leads to crossover designation and the establishment of both, crossover assurance and interference. However, studying how the synaptonemal complex regulates crossover formation remains challenging for most species since crossover formation typically depends on a fully assembled synaptonemal complex. Using Crispr/Cas9 mutagenesis, imaging and automated image analysis, we identified the C-terminus of the synaptonemal complex protein SYP-4 as the critical component regulating crossover formation in *C. elegans*. A C-terminally truncated SYP-4 is fully functional for synaptonemal complex assembly but both, crossover assurance and interference are impaired. These defects in crossover regulation in the C-terminally truncated SYP-4 mutant are caused by the mis-localization of the pro-crossover factor ZHP-3: ZHP-3 is absent from the synaptonemal complex but remains co-localized with the crossover marker COSA-1 suggesting that crossovers are formed. Together, our data demonstrate that the C-terminus of

SYP-4 recruits ZHP-3 to the synaptonemal complex, and the proper localization of ZHP-3 is required to establish both, crossover assurance and interference. Importantly, our data validate findings from plants demonstrating that the localization of the ZHP-3 homolog Hei10 to the synaptonemal complex is required for robust crossover regulation further supporting the coarsening model.

45 **PLK-1/Polo-like kinase is required to protect apicobasal polarity during mitosis in intestinal epithelia** Maria D Sallee, Jessica L FeldmanBiology, Stanford University

The ability of epithelia to line our organs and protect our bodies requires continued epithelial integrity through assaults like cell division. Tissue integrity relies on correct cell polarity and attachment, with apical surfaces facing the lumens of tube- and sac-shaped organs, basal surfaces facing the basement membrane, and junctional complexes adhering neighboring cells. However, cell division challenges this integrity during development and homeostasis. As epithelial cells divide, their polarized structures are remodeled: new junctions are built, the apical surface is cleaved, and interphase microtubule arrays originating from the apical surface are transiently removed as the mitotic spindle forms and are rebuilt upon mitotic exit. Using the developing *C. elegans* intestine as an *in vivo* epithelial model, we previously found that the apical PAR polarity complex ("PARs") protects apicobasal polarity and microtubule reorganization as cells divide (Sallee *et al.,* 2021), but how epithelial remodeling is coordinated with the cell cycle was unknown. We hypothesized that conserved mitotic kinases, which are critical for building the mitotic spindle, play an additional role in remodeling the apical surface and removing apical microtubules during mitosis. Thus, microtubules should remain at the apical surface in dividing cells in the absence of mitotic kinases. In contrast to this prediction, apical microtubules were still removed in mitosis upon intestine-specific depletion ("gut(-)") of the mitotic kinases PLK-1/Polo or AIR-1/Aurora A. Kinase-depleted cells experienced a prolonged mitosis and prolonged loss of apical microtubules, with some cells failing to rebuild apical microtubules upon mitotic exit. As the PARs function upstream of microtubule reorganization in dividing cells, we asked if the persistent loss of apical microtubules could be caused by loss of the PARs at the apical surface. Indeed, we observed that dividing PLK-1gut(-) cells formed gaps in apical PAR localization. As a result, many PLK-1gut(-) larval intestines formed dead-end lumens that failed to connect to the rectum. Surprisingly, AIR-1gut(-) intestines did not share the defects in PAR complex localization and lumen formation, suggesting a PLK-1-specific role in protecting both apicobasal polarity during mitosis and tissue integrity during organ formation. NIH NIGMS K99

46 **Intermediate filament network perturbation in the *C. elegans* intestine causes systemic dysfunctions** Florian Geisler[1], Sanne Remmelzwaal[2], Vera Jankowski[3], Mike Boxem[2], Rudolf E. Leube[11]Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany, [2]Division of Developmental Biology, Institute of Biodynamics and Biocomplexity, Department of Biology, Faculty of Science, Utrecht University, Utrecht, The Netherlands, [3]Institute for Molecular Cardiovascular Research, University Hospital RWTH Aachen, Aachen, Germany

Intermediate filaments (IFs) are major components of the metazoan cytoskeleton. A long-standing debate concerns the question whether IF network organization only reflects or also determines cell and tissue function. Using *C. elegans*, we have recently described mutants of the MAPK SMA-5, which perturb the organization of the intestinal IF cytoskeleton resulting in luminal widening and cytoplasmic invaginations. Besides these structural phenotypes, systemic dysfunctions were also observed. We now identify the IF polypeptide IFB-2 as a highly efficient suppressor of both the structural and functional deficiencies by removing the aberrant IF network. Mechanistically, perturbed IF network morphogenesis is linked to hyperphosphorylation of multiple sites throughout the entire IFB-2 molecule. The rescuing capability is IF isotype-specific and not restricted to SMA-5 mutants but extends to other regulators of IF network morphogenesis, i.e. the cytoskeletal linker IFO-1 and the IF-associated protein BBLN1. The findings provide strong evidence for adverse consequences of the deranged IF networks with implications for diseases that are characterized by altered IF network organization.

47 **More than a loading control: actin form and function during aging.** Gilberto Garcia, Maxim Averbukh, Naibedya Dutta, Toni Castro Torres, Darius Moaddeli, Athena Alcala, Sally Hoang, Max Thorwald, Ryo Higuchi-SanabriaUniversity of Southern California

The actin cytoskeleton is a three-dimensional scaffold of proteins that is a regulatory, energy-consuming network with dynamic properties to shape the structure and function of the cell. Proper actin function is required for many cellular pathways, including cell division, autophagy, chaperone function, endocytosis, and exocytosis. Deterioration of these processes manifests during aging and exposure to stress, which is in part due to the breakdown of the actin cytoskeleton. However, the regulatory mechanisms involved in preservation of cytoskeletal form and function are not well understood. Thus, we performed a multi-pronged, cross-organismal screen combining a whole-genome CRISPR-Cas9 screen in human fibroblasts with *in vivo C. elegans* synthetic lethality screening to identify novel regulators of actin health. We identified the bromodomain protein, BET-1, as a key regulator of actin function and longevity. Overexpression of *bet-1* preserves actin function at late age and promotes lifespan and healthspan in *C. elegans.* These beneficial effects are mediated through actin preservation by the transcriptional regulator function of BET-1.

Interestingly, we also find that BRD4 (homolog of *bet-1*) is required for senescent cell survival. Together, our discovery assigns a key role for BET-1 in cytoskeletal health, highlighting regulatory cellular networks promoting cytoskeletal homeostasis.

48 **AMPK determines small RNA pathway prevalence though Dicer reallocation to enhance microRNA synthesis and mediate soma-to-germ line communication** Elena Jurczak[1], Christopher Wong[2], Fabian Braukmann[3], Eric Miska[3], Richard Roy-[21]Biology, McGill University, [2]McGill University, [3]Cambridge University

Cells talk; it is well established that most cells can communicate with each other via signaling molecules and pathways to respond to both intrinsic and extrinsic cues. Until relatively recently, however, it was deemed impossible for this transfer of information to breach the soma-to-germ line barrier and for an organism's offspring to retain a molecular memory of life history events. Through our analyses, we have uncovered previously undescribed mechanisms that challenge this view by defining a role for somatic microRNAs in altering the molecular profile of germline stem cells to protect their reproductive integrity during periods of duress.

For many organisms, the energy sensor and master metabolic regulator AMPK maintains the homeostatic control of cellular energy balance by perceiving changes in resource availability and generating context-appropriate responses. *Caenorhabditis elegans* larvae exposed to various energetic stressors enter a stage of developmental quiescence (termed "dauer") which allows them to preserve cellular integrity when subjected to intervals of suboptimal growth conditions. Animals lacking AMPK signaling, however, exhibit post-dauer germline defects, aberrant gene expression changes, and drastically altered expression levels of small noncoding RNAs.

In this study, we show that AMPK regulates the quiescence of germline stem cells non-autonomously by directly altering the dynamics of small RNA biogenesis in the neurons. AMPK-mediated phosphorylation acts as molecular switch that drives the re-allocation of key enzymatic resources – notably that of the endonuclease Dicer and of its binding partners – to the microRNA biosynthesis pathway at the onset of the dauer stage. Correcting the expression levels of somatic microRNAs and regulating their association with secretory vesicles suppresses the germline defects of AMPK mutants. Thus, we demonstrate a mechanism through which this kinase bridges the gap between the soma and the germ line by fine-tuning the production of a population of somatic microRNAs that act as a "pro-quiescence" signal to maintain the integrity of the germ line during periods of extended energy stress.

49 **The role of chromatin factors in small-RNA-mediated, germline gene expression in *C. elegans*** Mindy Clark[1], Gloria Ha[1], Margaret Starostik[1], Jessica A Kirshner[1], Natasha E Weiser[1], Suhua Feng[2], John Yates III[3], Steven E Jacobsen[2], John K Kim-[11]Biology, Johns Hopkins University, [2]UCLA, [3]The Scripps Research Institute

Two opposing endogenous small RNA (endo-siRNA) pathways regulate gene expression and chromatin architecture in the *C. elegans* germline. The canonical germline endo-siRNA pathway silences thousands of gene targets and maintains this silencing across generations via H3K9me3 deposition. In contrast, the CSR-1 endo-siRNA pathway largely promotes gene expression of germline targets that are co-transcriptionally marked with H3K36me3. Loss of the *csr-1* Argonaute leads to sterility. Yet, how CSR-1 endo-siRNAs license gene expression remains largely unknown. Current work in the lab suggests that CSR-1 downregulates expression of the highly conserved chromatin factor, MORC-1, which functions in the germline to dampen expression of germline expressed genes. Thus, CSR-1 may license germline gene expression in part by attenuating MORC-1 expression. Here, we further explore two aspects of germline gene regulation. First, while MORC-1 binds at the transcriptional start sites of CSR-1-targeted genes, we show that MRG-1, a chromodomain-containing, putative H3K36me3 reader, binds along the gene bodies of these shared targets. MRG-1 and CSR-1 also physically interact, and CSR-1 regulates MRG-1 target binding and localization within germline nuclei. In turn, preliminary work suggests MRG-1 is required for MORC-1 binding at germline active genes, indicating that MRG-1 may be important for ensuring the fidelity of MORC-1 association with target loci. Second, because MORC-1 has the capacity to phase separate and form biomolecular condensates, we hypothesize that phosphorylation plays an important role in its function and assembly on chromatin via phase separation. MORC-1 is highly phosphorylated in the developing and adult germline, and our MORC-1 phospho-mass spectrometry analysis has identified 8 strongly phosphorylated residues in a conserved intrinsically disordered region. Protein kinase CK2 is required for MORC-1 phosphorylation, and germline-specific depletion of CK2 alters MORC-1 nuclear localization. Taken together, our study sheds light into the molecular mechanisms by which the chromatin factors MORC-1 and MRG-1 work together with CSR-1 to faithfully maintain the heterochromatin-euchromatin landscape of the germline genome.

50 **Genetic conflict and piRNAs drive the evolution of parent-of-origin gene expression** Pinelopi Pliota[1], Hana Marvanova[1], Alevtina Koreshova[1], Yotam Kaufman[2], Polina Tikanova[1], Daniel Krogull[1], Andreas Hagmüller[1], Sonya A Widen[1], Dominik Handler[1], Joseph Gokcezade[1], Peter Duchek[1], Julius Brennecke[1], Eyal Ben-David[2], Alejandro Burga[11]IMBA, [2]Hebrew University

Genomic imprinting—the nonequivalence of maternal and paternal genomes—is a critical process that has independently

evolved in numerous plant and mammalian species, including humans. According to kinship theory, imprinting is the inevitable consequence of conflictive selective forces acting on differentially expressed parental alleles. Yet, how these epigenetic differences evolve in the first place is poorly understood. Here we report the discovery and molecular dissection of a novel parent-of-origin effect on gene expression that illuminates this fundamental question. Toxin-antidote elements (TAs) are widespread selfish genes that increase their frequency in populations by poisoning non-carrier individuals. In reciprocal crosses between two *Caenorhabditis tropicalis* isolates from the Caribbean, we found that the *slow-1/grow-1* TA is specifically inactive when paternally inherited. This parent-of-origin effect stems from epigenetic repression of the *slow-1* toxin in the germline of heterozygous mothers. We show that reduction of Piwi Argonaute activity or deletion of two piRNA loci complementary to the 3'UTR of *slow-1* restores the toxicity of the selfish TA following its paternal inheritance. Remarkably, when *slow-1/grow-1* is maternally inherited, *slow-1* repression is halted by a translation-independent role of its maternal mRNA. That is, *slow-1* transcripts loaded into eggs prior to fertilization—but not SLOW-1 protein—are necessary and sufficient to counteract piRNA-mediated repression in the zygote. A related but divergent TA, *slow-2/grow-2,* does not show a parent-of-origin effect, indicating that this is a fast-evolving trait. Our findings show that parent-of-origin effects can evolve by co-option of the piRNA pathway, cementing the evolutionary link between parent-specific gene expression and host defense mechanisms beyond DNA methylation. Lastly, our results indicate that such parent-of-origin effects can provide a strong selective advantage: they hinder the spread of selfish genes that require sex for their propagation. We propose that parasitic conflict is a key evolutionary force fueling the emergence of imprinting.

51 **Genetic interaction screens reveal functional relationships between constitutive heterochromatin, the nucleolus, and the integrated stress response** Roopali Pradhan, Anna F Townley, Ana Dopico-Fernandez, Julie AhringerThe Gurdon Institute, University of Cambridge

H3K9 methylated constitutive heterochromatin is an important epigenetic compartment in the nucleus that is enriched at the nuclear and nucleolar peripheries. Heterochromatin-induced gene silencing and repetitive element repression is crucial for normal development, and defects in heterochromatin have been linked to many human diseases including cancer. However, the mechanisms through which heterochromatin is established and maintained are not well-understood in higher organisms.

We have built a comprehensive genetic interaction network to identify and functionally dissect novel components of constitutive heterochromatin in *C. elegans*. We carried out genetic interaction screens in seven heterochromatin mutant strains using RNAi and discovered genes whose knockdown enhanced or suppressed their phenotypes: H3K9 methyltransferases *(met-2* and *set-25)*, HP1 orthologues (*hpl-1* and *hpl-2),* and HPL-2 interactors (*lin-61, lin-13,* and *tdp-1)*. The screens revealed 289 enhancers, among which ~10% encode genes involved in nucleolar processes such as ribosome biogenesis. Other large classes include components of chromatin remodelling, protein modification, and RNA regulation pathways. Of the 89 suppressors, the largest group encodes proteins associated with active transcription. Most hits have a human orthologue and interact with more than one mutant strain, highlighting the high interconnectivity of the network. See the poster by Townley et al for more information about the screen.

Investigating the genetic interaction with nucleolar processes, we found that *hpl*-2 mutants have small nucleoli and are hypersensitive to the inhibition of protein translation. Mutants also show increased phosphorylation of eIF2alpha, indicating hyperactivation of the Integrated Stress Response (ISR) – a pathway that downregulates translation in response to proteotoxic stress. Preventing ISR induction through mutating the relevant site in eIF2alpha (S51A) led to suppression of the small nucleolar size of *hpl-2* mutants but an enhancement of the growth and fertility defects, and suggesting that a smaller nucleolus and ISR induction is protective. Our results suggest that activation of the proteotoxic stress response underlies major physiological consequences of heterochromatin dysfunction. Further analyses of the heterochromatin genetic network will aid understanding of heterochromatin function and dysfunction in metazoans.

52 **Histone H2A mono-ubiquitylation functions independently of Histone H3K27-trimethylation to regulate embryonic enhancers** Kailynn MacGillivray, Daniel Fusca, Luomeng Tan, Reta Aram, Arneet SaltzmanCell and Systems Biology, University of Toronto

Polycomb complexes are major regulators of chromatin state. Polycomb Repressive Complex 1 (PRC1) is responsible for depositing histone H2A lysine 119 mono-ubiquitylation (H2AK119ub), a post-translational histone modification that is believed to work cooperatively with PRC2-mediated histone H3 lysine 27 trimethylation (H3K27me3) to repress gene expression. In mice and *Drosophila,* maintenance of H2AK119ub or H3K27me3 enriched domains are dependent on the activity of both complexes. In *C. elegans*, mutations impacting putative PRC1 component homologs, *mig-32* and *spat-3*, and PRC2 homologs are associated with different developmental phenotypes, suggesting they play at least partially distinct roles. Thus, the extent to which *C. elegans* Polycomb complexes and their corresponding histone modifications function together or independently is uncertain. ChIP-seq profiling in wildtype and *mig-32* mutant embryos, revealed that the majority of H2AK119ub and H3K27me3 peaks were

distinct, with co-enrichment at only a subset of genes. Furthermore, we observed global loss of H2AK119ub in *mig-32* mutants, whereas the distribution of H3K27me3 was not significantly affected, suggesting that H2AK119ub and H3K27me3 are regulated independently in embryos. PRC1-like mutants also displayed variable, delayed developmental timing into adulthood, a phenotype that is rescued when the PRC2 complex is disrupted in tandem, suggesting that the activity of the two complexes may be interdependent post-embryogenesis. We are currently investigating if there are any unique transcriptional changes following combinatorial depletion of the PRC1-like and PRC2 complexes. Instead of colocalizing with H3K27me3, we found that in wild-type embryos, H2AK119ub colocalizes with H3K4me1, particularly at predicted repressed enhancers. We discovered that 72% of the genes putatively modulated by the H2AK119ub-enriched repressed enhancers are up-regulated in H2AK119ub-deficient mutants, suggesting a role for this histone modification in constraint of enhancer activity. The up-regulated genes are involved in nervous system development and functionality, potentially contributing to the previously observed defects in neuron migration and axon guidance in *mig-32* and *spat-3* mutants. Together, our results identify a previously unappreciated H3K27me3-independent role for H2AK119ub in the regulation of embryonic enhancers.

53 **E3 ubiquitin ligase ZSWIM8/EBAX-1 regulates microRNAs in a seed-dependent manner** Acadia L Grimme[1,2], Bridget F Donnelly[1,2], Katherine McJunkin[1]1National Institutes of Health, [2]Johns Hopkins

MicroRNAs (miRNA) are small RNAs that are loaded into an Argonaute protein to form an RNA-induced silencing complex (RISC) that post-transcriptionally regulates gene expression. While the regulatory role of miRNAs requires tight control of miRNA abundance, how the abundance of miRNAs is regulated, particularly at the level of decay, is not fully understood. In *C. elegans*, the *mir-35* family is essential for proper embryonic development and is tightly down-regulated in the embryo to larval transition (EtoL1). Our lab previously demonstrated that *mir-35* undergoes a two-phase regulation with embryonic abundance dependent upon the 3' end sequence of *mir-35* and EtoL1 decay dependent upon the 5' "seed" sequence (nucleotides 2-7). I have investigated the relationship between these two phases of *mir-35*regulation and a miRNA decay mechanism (target-directed microRNA degradation (TDMD)). In TDMD, extensive base pairing of a miRNA's 5' seed sequence and 3' end with a TDMD trigger RNA leads to the degradation of that miRNA. Thus, *mir-35* regulation differs from canonical TDMD because each phase requires only the 5' or 3' sequences, not both. Furthermore, I demonstrate that the 3' end regulation of embryonic abundance is not dependent on the TDMD effector (ZSWIM8/EBAX-1). However, the 5' seed sequence-dependent EtoL1 decay is dependent on EBAX-1, suggesting a mechanism related to TDMD wherein EBAX-1 triggers the degradation of RISC via Argonaute ubiquitination. This is notably different from the current model of ZSWIM8/EBAX-1-driven TDMD which requires the extensive pairing of the 3' end to drive decay. Because multiple Argonaute paralogs load miRNAs, loading into a specific Argonaute protein may contribute to this EtoL1 decay mechanism. While *mir-35* is preferentially loaded into the Argonaute protein ALG-2, I demonstrate that the EtoL1 decay is not dependent on this protein. Additionally, I developed a genetic screen to find other factors that drive *mir-35* degradation. Understanding how the *mir-35* family is regulated will shed light on the diversity of TDMD and related mechanisms.

54 **Lipid kinase PPK-1/PIP5K1A regulates microRNA biogenesis through interacting with nuclear export protein XPO-1/XPO5** Chun Li, Frank SlackBIDMC/Harvard Medical School

MicroRNAs (miRNAs) are small non-coding RNAs, which were first discovered through developmental studies in *Caenorhabditis elegans* (*C. elegans*). The *let-7* miRNA is highly conserved in sequence, biogenesis and function from *C. elegans* to humans. During miRNA biogenesis, XPO5-mediated nuclear export of pre-miRNAs is a rate-limiting step and, therefore, might be critical for the quantitative control of miRNA levels, yet little is known about how this is regulated. In *C. elegans,* the *lin-28/let-7* heterochronic pathway regulates the strict development timing of seam cells. Here we show that lipid kinase PPK-1/PIP5K1A (phosphatidylinositol-4-phosphate 5-kinase) directly regulates miRNA biogenesis. We found that *C. elegans* PPK-1 associates with nuclear export receptor XPO-1 both *in vitro* and *in vivo* and regulates miRNA expression and functions in the *lin-28/let-7* pathway. In human cells, we showed a conserved function of PIP5K1A (the ortholog of PPK-1), which interacts with nuclear export protein XPO5 in the nucleus to regulate miRNA levels in a kinase-independent manner. Furthermore, we demonstrated that PIP5K1A blocks the binding of XPO5 to pre-miRNA. Our study uncovers the novel finding of a direct connection between lipid kinase PPK-1/PIP5K1A and miRNA biogenesis. Given that miRNAs are implicated in multiple diseases, including cancer, this new finding might reveal a promising therapeutic avenue.

55 **tRNA-fragments in sperm regulate post-fertilization embryonic gene expression and offspring phenotypes** Olivia Crocker[1], Nicholas Galambos[2], Colin Conine[1]1University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia, [2]Biology, University of Pennsylvania

The environment encountered by an organism can modulate epigenetic information in the gametes to transmit non-genetically inherited phenotypes to offspring. In mice, the diet of male mice regulates specific tRNA-fragments (tRFs) in sperm, which after fertilization regulate embryonic gene expression and through uncharacterized changes in development generate metabolic phenotypes in offspring. Here we demonstrate that *C. elegans* sperm also accumulates tRFs in their sperm that are regulated

by the environment and can transmit epigenetically inherited phenotype to offspring. Regulation occurs through the RNaseT2 enzyme *rnst-2* which processes cleaved tRNA-halves into shorter fragments for recycling in the lysosome. The regulation of this processing produces tRFs in sperm which after fertilization regulate early embryonic and developmental gene expression and further, elicit adaptive phenotypes in progeny. This work establishes tRFs as a deeply conserved carrier of intergenerational epigenetic information and the worm as a model for dissecting this non-genetic inheritance mechanistically.

56 **N-terminal processing of Argonaute proteins affects epigenetic inheritance** Ida J. Isolehto, Jan Schreier, Svenja Hellmann, René F. KettingBiology of Non-coding RNA, Institute of Molecular Biology

C. elegans employs several small RNA mediated pathways to initiate and maintain proper gene regulation throughout development, and across generations. These pathways rely on Argonaute proteins (AGOs) that perform target silencing. Despite being well-studied pathways, little is known about how the AGOs themselves are regulated. In germ cells, small RNAs and associated AGOs are particularly important to provide genome stability and maintain an immortal germline. Strikingly, silencing responses can become independent of the triggering small RNAs and maintained across generations through epigenetic inheritance.

Several Worm specific Argonautes (WAGOs) have a Proline rich N-terminal intrinsically disordered region (IDR) and it has been shown that the N-terminus of WAGO-1 and WAGO-3 are being processed by the dipeptidyl aminopeptidase DPF-3 *in vivo*[1]. Processing by DPF-3 is required for transposon silencing, fertility, and correct small RNA loading[1]. This presents an important role for N-terminal processing in WAGO function. However, based on the DPF-3 cleavage pattern and amino acid specificity, DPF-3 cannot be solely responsible for the fully processed WAGO-1/3 N-terminus. We identified another peptidase, the X-prolyl aminopeptidase APP-1[2], that also has the potential to cleave the WAGO-1/3 N-terminus.

We generated *app-1* mutants, performed genetic experiments, and found that similarly to DPF-3[1], APP-1 is required for silencing of transposons. Additionally, we discovered that both *app-1* and *dpf-3* are crucial for RNAi inheritance as both these mutants completely fail to inherit RNAi. We found that both APP-1 and DPF-3 localize to PEI-granules in spermatids, which we identified as germ granules that are crucial for paternal RNAi inheritance via WAGO-3 [3]. To understand if peptidase activity affects WAGO-3 localization, we tagged WAGO-3 with GFP internally to avoid interference with N-terminal processing and found that APP-1 and DPF-3 affects proper WAGO-3 localization to PEI-granules. We are now further investigating the role of N-terminal processing in Argonaute function and epigenetic inheritance.

[1]Gudipati *et al.* Mol Cell, 2021. [2]Placentino *et al.* EMBO J, 2021. [3]Schreier *et al.* Nat Cell Biol, 2022.

57 **Nucleus-Independent Transgenerational RNAi inheritance in *C. elegans*** Itai Rieger[1], Guy Weintraub[1], Itamar Lev[1], Dana Bar-Zvi[1], Sarit Anava[1], Hila Gingold[1], Shai Shaham[2], Oded Rechavi[11]Tel Aviv University, [2]The Rockefeller University

in contrast to many experts' opinions and contradicting one of the main dogmas in biology, in the last 25 years research on *C. elegans* nematodes demonstrated that animals can transmit parental responses transgenerationally. In worms, ancestral RNA interference (RNAi) responses regulate gene expression for many generations, without changing the DNA sequence. Nevertheless, there are still many unknown questions; it remains unclear whether the primary agent that perpetuates heritable silencing in nematodes is RNA or closed chromatin states, and whether the information is communicated to the next generation inside or outside of the nucleus. Here we use the simplicity and tractability of gene-specific dsRNA-induced heritable silencing to answer these questions. We demonstrate that RNAi can be inherited independently of chromatin or any other nuclear factors, from mothers that are genetically engineered to only transmit their cytoplasm but not their nuclei, and thus not their DNA and chromatin, to the next generation. Nucleus-independent RNA inheritance depends on cytoplasmic germ granule proteins, and can be potentiated by disturbing proper germ granules segregation. Further, utilizing sequence polymorphism between different *C. elegans* isolates and using small RNA and mRNA sequencing we identify and study numerous endogenous small RNAs which are inherited in a nucleus-independent manner. Together, our results suggest that unlike the animal's genome, epigenetic information is also transmitted via small RNAs in the germline's cytoplasm, and that chromatin marks are not required for transgenerational RNAi inheritance.

58 **Unbiased forward genetics reveals novel mechanisms of transgenerational inheritance** Shiela Pearl Quiobe, Ralf J SommerIntegrative Evolutionary Biology, Max Planck Institute for Biology

Environmental cues can have profound effects on organismal development and phenotype, often regulated by small RNAs. We have used long-term environmental induction experiments to study the influence of shifts in microbial diet on mouthform plasticity in *Pristionchus pacificus.* This nematode exhibits a mouth dimorphism with the eurystomatous (Eu) form being a potential predator on nematodes, whereas the stenostomatous (St) form is a strict bacterial feeder. We used a wild isolate of *P. pacificus* that is preferentially St on *E. coli* OP50. Directed evolution of 110 genetically identical lines for 101 generations on a *Novosphingobium* diet revealed immediate and systemic diet-induced plasticity, resulting exclusively in the formation of the Eu

morph. Periodic diet-reversals to OP50 starting in F15, F25 etc revealed transgenerational memory that entails multigenerational plasticity. We combined these long-term induction experiments with unbiased forward genetic screens by performing an EMS mutagenesis in generation F14 on *Novosphingobium* to find mutants defective in transgenerational inheritance. From a screen of 9,900 'F2' progeny of mutagenized animals, we found 165 potential candidates for *t*ransgenerational *i*nheritance *d*efective (*tid*) after food reversal. Whole genome sequencing revealed that multiple candidate genes were hit several times independently (*i.e.* >3 alleles). Indeed, generating clean CRISPR mutants in the first five *tid* candidate genes revealed a *tid* phenotype after diet reversal from *Novosphingobium* to OP50. We will describe novel molecular mechanisms and epigenetic factors identified through the analysis of these *tid* mutants, providing novel insight into transgenerational inheritance and its conservation in *C. elegans*.

59 **P bodies coat germ granules to promote transgenerational gene silencing in *C. elegans*** Zhenzhen Du[1], Kun Shi[1], Jordan Brown[2], Tao He[1], Wei-Sheng Wu[3], Ying Zhang[1], Heng-Chi Lee[2], Donglei Zhang[1,21]Biochemistry and Molecular Biology, Huazhong University of Science and Technology, [2]Molecular Genetics and Cell Biology, University of Chicago, [3]Electrical Engineering, National Cheng Kung University

The formation of biomolecular condensates has emerged as a critical mechanism for compartmentation in living cells. Despite interactions between distinct condensates having been reported, the biological relevance of these interactions remains elusive. In germ cells, small RNA silencing factors are enriched in germ granule condensates, where distinct factors are organized into sub-compartments with specific functions linked to genome surveillance or transgenerational gene silencing[1-3]. Here we showed that P body condensates, which are known for housing translationally-inactive mRNAs and mRNA degradation factors[4,5], are situated specifically at the cytoplasmic side of perinuclear germ granules. Disruption of P body factors, including CGH-1/DDX6 and CAR-1/LSM14, lead to dispersal of small RNA factors from perinuclear germ granules and disorganization of sub-compartments within germ granules. We further found that CAR-1 promotes the interaction between CGH-1 and germ granule factors, and these interactions are critical for CGH-1's ability to promote piRNA-mediated gene silencing. Importantly, we observed that *cgh-1* mutants are competent in triggering gene silencing but exhibit defects in maintaining gene silencing in subsequent generations. We trace this loss of transgenerational silencing to defects in amplifying secondary small RNAs and the degradation of WAGO-4 Argonaute, both known carriers of gene silencing memories. Together, our results uncover the function of P body factors in small RNA-mediated transgenerational gene silencing and highlight how the formation and function of one condensate can be regulated by an adjacent, interacting condensate in cells.

1. Ishidate et al., Molecular Cell (2018); 2. Wan et al., Nature (2018); 3, Xu et al., Cell Reports (2018); 4. Noble et al., JCB (2008); 5, Cassani et al., Development (2022)

60 **Analysis of WAGO-1(Y613E) reveals specialization of GLH paralogs for promoting Argonaute loading and localization within nuage** Humberto J. Ochoa, Daniel J. Durning, Siyuan Dai, Craig C. MelloUniversity of Massachusetts Medical

A key event in the life of an Argonaute is acquisition of the appropriate guide RNA (gRNA). To identify factors that promote WAGO-1 loading we mutated tyrosine 613 to glutamate (Y613E), a lesion predicted to prevent gRNA loading. While this lesion rendered WAGO-1 nonfunctional, as expected, we were surprised to observe an array of cytological defects within the nuage (P granules) where WAGO-1 protein normally resides. Specifically, we found that WAGO-1(Y613E) lost its P granule enrichment and instead was diffusely localized in the cytoplasm. The DEAD-box helicase protein GLH-1 which along with its paralog GLH-4 interacts with WAGO-1, also exhibited reduced P-granule localization. The GLH-1 ATP binding site mutant GLH-1(K391A), which is known to bind WAGO-1 more tightly, caused enhanced cytoplasmic re-localization of GLH-1(K391A), suggesting that WAGO-1(Y613E) sequesters GLH-1 from the nuage.

Wago-1(Y613E) did not disrupt the localization of GLH-2, GLH-3, PGL-1, CSR-1, and ZNFX-1 from nuage. However, strikingly, we found that GLH-4 and the PRG- 1 Argonaute which both normally localize broadly within P granules, instead re-localize to a single domain of nuage associated with each germline nucleus. Co-staining experiments revealed that this GLH-4 PRG-1 nuage domain localizes proximal to the intranuclear piRNA-cluster transcription sites.

GLH-1 and GLH-4 bind to WAGO target RNAs but also bind to piRNAs, which make up 25% of the RNA species identified in GLH-4 Cross-Linking and IP (CLIP) studies, and 5% of reads associated with GLH-1. The proportion of unprocessed piRNA precursors relative to mature piRNAs increased in Y613E. Processing of piRNAs involves trimming of the 3' end, and interestingly a GLH-1 lesion that blocks ATP hydrolysis caused GLH-1 CLIP to recover over trimmed piRNAs. We hypothesize that GLH-1 and GLH-4 may bind directly to piRNAs to prevent them for trimming, and that this event becomes delayed in the context of Y613E. The enlarged domain of GLH-4 and PRG-1 nuage may therefore reflect a bottleneck in piRNA processing perhaps because factors shared between WAGO and PRG-1 loading are sequestered in the Y613E mutant. In summary, these findings reveal a remarkable dynamic of molecular activity within nuage and point to specialization among GLH family members in promoting WAGO-1 and

PRG-1 loading.

61 **Emergence of whole brain axon-axon patterning from early collective cell behaviors** Christopher Brittin[1], Anthony Santella[2], Kristopher Barnes[2], Mark W Moyle[3], Li Fan[2], Ryan Christensen[4], Irina Kolotuev[5], William A Mohler[6], Hari Shroff[4], Daniel Colón-Ramos[3], Zhirong Bao[11]Memorial Sloan Kettering Cancer Center, [2]Memorial Sloan Ketterning Cancer Center, [3]Yale University School of Medicine, [4]Janelia Research Campus, Howard Hughes Medical Institute, [5]Université de Lausanne, [6]University of Connecticut Health Center

Axon-axon patterning is the stereotyped and flexible spatial arrangement of axons that supports brain organization. The *C. elegans* nerve ring exhibits two key axon-axon patterning features: stereotyped sorting of axons into spatial domains and variance of pairwise axon-axon placement across individuals (neurovariability). By merging dynamic single-cell tracking of tissue morphogenesis in the *C. elegans* embryo with spatially-resolved electron micrograph data of the nascent nerve ring, we map how axon-axon patterning emerges through three sequential phases of collective cell behaviors, with each phase providing additional organization refinement. Phase 1: A chain of multicellular rosettes spatially sorts primordial neurons along the future nerve ring path. Phase 2: Rosette centers induce neuronal polarization and project pioneer fascicles along tissue substrates where (as confirmed by EM) the rosette fascicles converge into an ordering that corresponds to the nerve ring's spatial domains. SAX-3/Robo influences both rosette formation and fascicle outgrowth, suggesting possible roles in tissue cohesion and/or neuronal polarization. Phase 3: Follower axons intercalate between and are guided by pioneer fascicles. By engineering an *in silico* simulator of nerve ring development, we show that the two features of axon-axon patterning – domain sorting and neurovariability – naturally emerge when pioneers provide a centralized organizing substrate to regulate contact among followers during intercalation. Our findings demonstrate how macro-level brain features emerge from the persistent coordination of collective cell behaviors.

62 **Mapping the neuropeptide signaling network and its evolution in nematodes** Luca Golinelli[1], Ellen Geens[1], Sven Zels[1], Elke Vandewyer[1], Olivier Mirabeau[2], Ciaran McCoy[3], Louise E Atkinson[3], Angela Mousley[3], Liliane Schoofs[1], Liesbet Temmerman[1], Isabel Beets[11]KU Leuven, [2]Institut Pasteur, [3]Queen's University Belfast

Neuropeptides are one of the largest groups of neural messengers and key regulators of behavior and physiology. *C. elegans* has at least 159 putative neuropeptide receptor genes and is estimated to produce more than double this number of mature neuropeptides. The vast number and expansions of peptide and receptor genes poses a challenge to understand the functional organization and evolution of neuropeptide signaling networks in nematodes. Nevertheless, insight into this signaling landscape is important to answer fundamental questions on the structure and evolutionary diversification of neural signaling networks, to characterize functions of neuropeptides in nematode biology, and to catalyze development of new anthelmintics.

To obtain a deeper understanding of the evolution of nematode neuropeptide systems, we performed a pan-phylum phylogenetic analysis of neuropeptide G protein-coupled receptors (NP-GPCRs), using proteomes of 125 nematodes representing seven clades. We found that out of 31 ancient bilaterian NP-GPCR families, 17 are highly conserved in nematodes. These include orthologs of well-known vertebrate receptor families, like tachykinin, gonadotropin-releasing hormone and galanin signaling systems. In addition, we discovered several novel NP-GPCR candidates and expansions of protostomian- and phylum-specific peptide receptors in nematodes. Among these we were able to identify significant differences in conservation across nematodes, shortlisting 22 highly conserved receptors that are likely to be central players in nematode physiology.

In parallel, we set up a large-scale *in vitro* screening platform to biochemically identify ligand-receptor interactions for neuropeptides and GPCRs (Beets et al., 2022 BioRxiv). In this pipeline we systematically screened all predicted *C. elegans* NP-GPCRs with a comprehensive peptide library. Using a reverse pharmacology approach, we found over 460 peptide-GPCR couples and identified neuropeptide ligands for 62 *C. elegans* NP-GPCRs, which more than doubles the number of deorphanized peptide receptors in nematodes. This also uncovered additional ligands for known peptide GPCRs and complex combinatorial interactions. Our work provides tools and insights to study coevolution of ligands and receptors across the whole nematode phylum. This permits to take species- and application-driven nuances, including but also reaching far beyond *C. elegans*, into account, when studying neuropeptidergic networks.

63 **Dissecting the Functional Organization of the C. *elegans* Serotonergic System at Whole-Brain Scale** Di Kang, Ugur Dag, Ijeoma Nwabudike, Matthew Gomes, Jungsoo Kim, Adam Atanas, Eric Bueno, Cassi Estrem, Sarah Pugliese, Ziyu Wang, Emma Towlson, Steven FlavellMassachusetts Institute of Technology

Serotonin influences many aspects of worm behavior. How serotonin acts on its diverse receptors across the brain to modulate global activity is unknown. Here, we examine how serotonin release in C. *elegans* alters brain-wide activity to induce foraging behaviors, such as slow locomotion and increased feeding. Comprehensive genetic analyses identify three core serotonin receptors (MOD-1, SER-4, LGC-50) that induce slow locomotion upon serotonin release, and three other serotonin receptors (SER-1, SER-5, SER-7) that interact with them to modulate the temporal dynamics of this behavior. Specifically, SER-4 induces

behavioral responses to sudden increases in serotonin release, whereas MOD-1 induces behavioral responses to persistent serotonin release. The behavioral effect of isolated MOD-1 or LGC-50 activation, as well as the interaction among the six receptors, can be altered by satiety. Whole-brain calcium imaging of freely moving animals reveals widespread serotonin-associated brain dynamics, spanning multiple behavioral networks. We map all sites of serotonin receptor expression in the connectome, which, together with synaptic connectivity, help to predict which neurons show serotonin-associated activity during foraging. These results reveal how serotonin acts at defined sites across a connectome to modulate brain-wide activity and behavior.

64 **Spiking neural circuit underlying the *C. elegans* gut-brain ultradian rhythm** Jingyuan Jiang[1], Yifan Su[1], Rulin Zhang[1], Haiwen Li[1], Minxian Peng[2], San Chun Chiu[2], Qiang Liu[31]Peking University, [2]City University of Hong Kong, [3]Neuroscience, City University of Hong Kong

Rhythmic behaviors governed by internal biological clocks couple physiology to the nervous system in all animals with periods ranging from milliseconds to a day to years. A particularly interesting and well-studied rhythmic behavior controlled by the intestine and the enteric nervous system is the defecation cycle in *Caenorhabditis elegans*. This rhythmic behavior, also called the defecation motor program (DMP), consists of a series of stereotyped motor sequences activated once every ~45 seconds when there is abundant food. Previous cell ablation studies defined functions for the GABAergic enteric motor neurons called AVL and DVB in the expulsion step of the defecation behavior, but the specific roles and their coordination remain unclear. Here we identified AVL and DVB as two spiking motor neurons that function in the spatial-temporal regulation of the defecation cycle by firing nearly synchronized action potentials. In particular, both neurons fire broad, all-or-none calcium-mediated action potentials under current-clamp recording, presumably for synchronizing downstream muscle contractions that lead to expulsion. Extraordinarily, AVL fires compound action potentials with each upward spike followed by a time-locked upside-down or negative spike. Ion substitution and mutant analysis indicate that upward spikes are initiated and maintained primarily by the voltage-gated calcium channel UNC-2, while the upside-down spikes are mediated by the repolarization-activated potassium channel EXP-2. Behavioral analysis and live calcium imaging experiments revealed that coordinated action potential firing in AVL and DVB medicated by UNC-1 gap junctions and the negative spikes in AVL are important for reliable expulsion behaviors. Altogether, our work identified a spiking neural circuit constituted by AVL and DVB underlying the *C. elegans*defecation motor program: AVL entrains DVB by firing intrinsically coupled calcium- and potassium-mediated action potentials followed by a long-lasting afterhyperpolarization (AHP), enabling the regulation of the rhythmic signals from the intestinal clock at multiple time scales.

65 **Sexual dimorphism of whole-brain responses to a broad chemical space** Maedeh Seyedolmohadesin[1], Xingyang Fu[2], Mahdi Torkashvand[1], Kevin W Rusch[2], Sina Rasouli[1], Frank C Schroeder[3], Eviatar Yemini[2], Vivek Venkatachalam[11]Northeastern University, [2]UMass Chan Medical School, [3]Cornell University

Sexually dimorphic brain circuits play a critical role in shaping sex-specific behaviors in many species. These circuits are responsible for integrating sensory inputs and modulating neural activity in response to external cues, ultimately leading to sex-specific behaviors. *C. elegans* males and hermaphrodites exhibit differences in their responses to environmental cues, such as food and pheromones. Previous studies suggest that the structural and functional differences in the nervous system play a crucial role in mediating sexually dimorphic behavior. A recently obtained male connectome has revealed substantial differences in neural wiring patterns between the two sexes. However, the extent to which functional connectivity and neuronal activity contribute to sexually dimorphic behavior is still not fully understood.

To address this, we developed a novel system that records whole-nervous-system activity in both males and hermaphrodites while presenting them with a diverse set of external sensory cues. With this system, we were able to characterize whole-brain responses to a panel of ethologically relevant chemical cues. Our system uses a modified confocal microscope with single-cell resolution that can image the worm's nervous system at up to 10 volumes/second. We used NeuroPAL to identify the individual neurons within the nervous systems of both sexes, and built microfluidic devices to accommodate both sexes, taking into account the morphological differences between males and hermaphrodites. These devices enable us to simultaneously record the neuronal activity of all head, tail, and most midbody neurons in response to stimuli. Moreover, our design enables us to sequentially present more than 10 chemical stimuli to the animal's nose, and we were able to repeat the entire sequence 3 times and observe the animal's response for over 30 minutes. This approach enables us to evaluate the variability in state-dependent responses to repeated exposure of the same stimulus. We studied concentration-dependent attractive and repulsive responses to a diverse set of stimuli including gustatory, olfactory, pheromone and nociceptive cues. Using these stimuli, we examined known dimorphisms in neural activity and also discovered a substantial set of previously unknown dimorphisms as well.

Our system determined stimulus-evoked responses in sensory, inter-, and motor neurons, recapitulating previous findings such as sexually dimorphic responses of the ADF-mediated food/pheromone pathway. We additionally found many novel sexually dimorphic responses with significant differences observed in the shape, amplitude, and kinetics of hermaphrodite versus male neural activity. Nevertheless, on a global scale we found pairwise correlations between individual neurons to be largely similar

between sexes, indicating the potential for a large degree of conserved functional connectivity.

66 **Kin-recognition and nepotism mediate collective behaviours in the cannibalistic nematode *Pristionchus pacificus*** Fumie Hiramatsu, James W. LightfootGenetics of Behavior, Max Planck Institute for Neurobiology of Behavior – caesar

Many organisms collectively aggregate with members of the same species which can influence their group dynamics, population structure and ecology. However, understanding the fundamental mechanisms which determine an organism's propensity to aggregate and additionally the composition and configuration of the collective is difficult due to the complex nature of these interactions. By exploring a highly aggregating clade of the nematode *Pristionchus pacificus*, we have investigated factors influencing its collective behaviours in a genetically tractable system. We conducted pairwise aggregation assays between distinct *P. pacificus* strains of differing genetic distance and observed a significant preference for between kin aggregations. In contrast, groupings between more distantly related strains were avoided. Moreover, when two distantly related strains interact, one dominates and aggregates while solitary behaviour is induced in the rival strain which subsequently endures less preferable conditions. To explore a mechanism for this group selectivity, we analysed *P. pacificus* predation behaviours further. *P. pacificus* kills other nematodes including con-specifics, however, a small peptide mediated kin-recognition system prevents attacks on close relatives. Accordingly, CRISPR/Cas9 induced mutations which abolish predatory behaviours result in rival strains successfully aggregating together. Furthermore, mutations in the essential kin-signalling component *self-1*, prevent efficient aggregation events between kin. Finally, as *Caenorhabditis elegans* are found naturally occurring with *P. pacificus,* we explored collective events between these species. Here, aggregates were dominated by *P. pacificus* with only a small number of these predators proving sufficient to disrupt *C. elegans* group dynamics and prevent their aggregation. Thus, aggregating strains of *P. pacificus* preferentially group with kin over more divergent strains, revealing competition and nepotism as previously unknown components influencing collective behaviours in nematodes.

67 **C. elegans sphingolipid metabolism and pathogen defense are modulated by a microbiota-derived sphinganine** Lena Peters[1], Moritz Drechsler[2], Barbara Pees[1], Georgia Angelidou[3], Liesa Salzer[4], Karlis Moors[5], Nicole Paczia[3], Hinrich Schulenburg[1], Christoph Kaleta[5], Michael Witting[4], Helge Bode[2], Katja Dierking[11]Evolutionary Ecology and Genetics, Zoological Institute, Christian-Albrechts-University, [2]Institut für Molekulare Biowissenschaften, Goethe University Frankfurt, [3]Core Facility for Metabolomics and Small Molecule Mass Spectrometry, Max Planck Institute for Terrestrial Microbiology, [4]Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, [5]Research Group Medical Systems Biology, University Hospital Schleswig-Holstein Campus Kiel

Although it is becoming increasingly clear that microbiota play an important role in protecting their host from infection, the underlying mechanisms of microbiota-mediated protection are still largely unknown. Here, we seek to understand the mechanisms by which the natural microbiota isolate *Pseudomonas fluorescens* MYb115 protects *C. elegans* against *Bacillus thuringiensis* (Bt), on both the worm and bacterial sides. On the bacterial side, we found that a MYb115 biosynthesis gene cluster encoding a type I polyketide synthase (PKS) produces three long chain sphinganine derivatives. We generated a non-sphinganine producing Δ*sgaAB* MYb115 mutant and show that sphinganine is required for MYb115 mediated protection against Bt. Sphinganines belong to the sphingolipids, a group of membrane and bioactive lipids that function in membrane integrity and dynamics, but also the regulation of apoptosis, cell differentiation and proliferation. On the worm side, we found that modulation of sphingolipid metabolism and ceramide levels of *C. elegans* strongly influence survival after Bt infection. Ceramidase (*asah-1/asah-2*) and sphingomyelin synthase (*sms-1*) mutants are more susceptible to infection, highlighting their importance for *C. elegans* defenses against Bt. Using transcriptomic and sphingolipidomic analyses of the *C. elegans* response to MYb115 and Δ*sgaAB* MYb115, we further demonstrate that MYb115-derived sphinganine affects host defense and sphingolipid metabolism. In particular MYb115 interferes with *C. elegans* sphingolipid metabolism at the conversion of dihydroceramide and ceramide to sphingomyelins and hexosylceramides. We are currently investigating which cellular processes are involved in microbiota-mediated protection downstream of sphingolipid metabolism. Overall, we identified a novel protective metabolite from the *C. elegans* natural microbiota and demonstrated the importance of microbiota-mediated modulations in *C. elegans* sphingolipid metabolism for protection against pathogens.

68 **Metabolism orchestrates direct reprogramming of germ cells to neuron-like cells** Amin R. Shadfar[1], Nida Ul Fatima[2,3], Ismail Özcan[4], Baris Tursun[51]Molecular Cell Biology, Institute of Cell and Systems Biology of Animals University of Hamburg, [2]School of Medical Sciences, University of New South Wales, Australia, [3]Molecular Cell Biology, Institute of Cell and Systems Biology of Animals, University Hamburg, [4]Molecular Cell Biology, Institute of Cell and Systems Biology of Animals, University of Hamburg, [5]Molecular Cell Biology, Institute of Cell and Systems Biology of Animals, University of Hamburg

Conversion of cells identities via expression of fate-determining transcription factors (TF) is well-known as cellular reprogramming and has mostly been investigated in mammalian tissue cultures. *C. elegans* provides *in vivo* studies of cell fate reprogramming to dissect the complex interplay of different physiological processes that are critical during fate conversion. We previously identified

that suppression of the mitochondrial isocitrate dehydrogenase IDHA-1 creates permissiveness for direct reprogramming of germ cells to neuron-like cells. This phenomenon is induced upon overexpression of the ZNF TF CHE-1 using a heat-shock promoter in conjunction with RNAi against *idha-1*. CHE-1 specifies the ASE gustatory neuron fate during development and its overexpression is capable of ectopically inducing the ASE neuron fate, which can be visualized using the ASER-specific reporter *gcy-5::gfp*.

To dissect the molecular cascades that extend from mitochondria to the nucleus upon IDHA-1 depletion and create permissiveness for inducing germline to neuronal cell reprogramming, we performed double RNA interference (RNAi) screens. Notably, depletion of the putative monosaccharide transporter FGT-2 significantly enhanced germ cell reprogramming. Furthermore, RNAi against the sodium transporter ZK822.5, anion transporter SULP-6, and the monosaccharide transporter Y37A1A.3, also affected germ cell reprogramming efficiencies. Together with our finding that depletion of IDHA-1 protein levels by using degron-mediated protein degradation in the somatic gonad results in enhanced germline reprogramming, these results suggest a cross-talk of metabolites and intermediates between tissues. This notion is further corroborated by metabolomics which pointed towards glutamine anaplerosis to replenish TCA a-Ketoglutarate levels in *idha-1* RNAi worms, which we confirmed also based on genetic assessment.

Overall, our data illustrate that shifts in the TCA cycle cause alterations in metabolism which trigger changes in the nuclear gene expression pattern resulting in permissiveness for germ cell reprogramming. A better understanding of metabolites and intermediates that act as signalling molecules to sense perturbed physiological processes is important. They may trigger gene expression changes indirectly or directly which has high relevance to different disciplines including stem cell and cancer cell biology research.

69 **MORC-1 is a key component of the *C. elegans* CSR-1 germline gene licensing mechanism** Jessica A. Kirshner[1], Colette Picard[2], Natasha Weiser[1], Suhua Feng[2], Sonia El Mouridi[3], Kai Inoki[1], Nicita Mehta[1], Christian Froekjaer Jensen[3], Steve E. Jacobsen[2], John K. Kim[11]Johns Hopkins University, [2]UCLA, [3]King Abdullah University of Science and Technology

Nuclear small RNA pathways perform the canonical function of silencing pseudogenes, transposons, and attenuating the expression of protein-coding genes in the *C. elegans* germline. An opposing pathway, mediated by a distinct subset of endogenous siRNAs (endo-siRNAs) that engage the essential Argonaute, CSR-1, is thought to promote transcription of germline genes. This atypical 'gene licensing' pathway is defined by the observation that targets of CSR-1 are downregulated in *csr-1* mutants by a poorly understood mechanism. Here, we present data that contribute to our understanding of the CSR-1-dependent germline gene licensing mechanism. Although CSR-1-mediated gene targeting correlates with the licensing of germline gene expression, CSR-1 also robustly cleaves and degrades a small subset (~3%) of its targets through its endonucleolytic 'Slicer' activity. One such target encodes MORC-1, a conserved DNA-binding protein that efficiently condenses DNA and chromatin (Kim, *et al*., *Mol Cell*, 2019). Mutations in *csr-1* result in *morc-1* de-repression and overexpression in the germline. ChIP-seq in purified germline nuclei reveals that MORC-1 binds to the transcriptional start sites (TSSs) of germline-expressed genes and significantly overlaps with CSR-1-licensed target genes. In *csr-1* mutants, MORC-1 overexpression leads to increased binding at the TSSs and ectopic spreading to coding sequences of the MORC-1/CSR-1 shared gene targets, as well as decreased mRNA expression from these loci. Conversely, in *morc-1* mutants, the expression of MORC-1/CSR-1 shared targets is specifically upregulated. Taken together, these data indicate that attenuation of *morc-1*expression by CSR-1 prevents MORC-1 overexpression and inappropriate silencing of genes expressed in the germline. In support of this model, we show that loss of *morc-1* partially rescues multiple defects of *csr-1* mutants, including changes in gene expression and histone modifications and animal sterility. Conversely, overexpression of MORC-1 alone induces potent animal sterility, reminiscent of *csr-1* defects. Collectively, our results support a model by which CSR-1-mediated regulation of *morc-1* plays a significant role in the germline gene licensing mechanism.

70 **Heterochromatin readers CEC-6 and CEC-3 regulate RNAi inheritance and genome-wide H3K9me3 distribution** Chengyin Li, Aly Muhammad Ladak, Phoebe A.W. Bhagoutie, Victor Lao, Arneet L. SaltzmanCell and Systems Biology, University of Toronto

Chromatin and small RNA pathways ensure proper transcription regulation of genes and repetitive elements, which is essential for both somatic development and maintenance of germline integrity. Transcriptionally active euchromatin and transcriptionally repressed heterochromatin are often associated with distinct post-translational histone modifications. The small RNA-mediated nuclear RNA interference (RNAi) pathway can induce both heritable co-transcriptional silencing and heterochromatinization at its target genomic loci. However, the roles of histone modifications and their interplay with RNAi in locus-specific and genome-wide regulation remain to be fully explored.

We previously found that *C. elegans* chromodomain proteins CEC-6 and CEC-3 can recognize heterochromatin-associated histone H3 lysine 27 and lysine 9 methylation (H3K27me and H3K9me). The deletion of these two *cec* genes led to a progressive loss of fertility, suggesting a role in transgenerational chromatin regulation. Using a single-copy germline *gfp* reporter, we found that *cec-*

6 and *cec-3* limit the transgenerational duration of RNAi-induced silencing. Prolonged RNAi inheritance in *cec-3;cec-6* mutants is associated with elevated H3K9me3 and H3K27me3 at the RNAi target, consistent with its silenced transcription state. Furthermore, we found that both germline and somatic expression of a repetitive *gfp* transgene are inhibited by *cec-6*. Interestingly, since *cec-6* is mainly expressed in the germline, this may involve germline-to-soma communication through small RNAs. The increased repetitive *gfp* transgene expression in *cec-3;cec-6* mutants also corresponds to reduced H3K9me3 and elevated H3K4me3 at the repetitive array. Additionally, our ChIP-seq data show that *cec-6* and *cec-3* promote H3K9me3 enrichment at heterochromatic regions and restrict H3K9me3 enrichment at euchromatic regions. In particular, these two *cec* genes promote H3K9me3 at LTR and LINE retrotransposons and at germline nuclear RNAi targets. We also observe reduced H3K9me3 in *cec-3;cec-6* mutants at genes upregulated in intracellular pathogen response, which may be a result of retrotransposon misregulation. Altogether, we propose that *cec-6* and *cec-3* regulate transcription and long-term preservation of the germline through heterochromatin and RNAi mechanisms.

71 **Mating strategy determines context-dependent sexual behavior** Eya Wolfson[1], Shachaf Shapira[1,2], Rizwanul Haque[1], Sonu Peedikayil-Kurien[1], Mattia Morandi[2], Irit Goldian[3], Elena Fidel[4], Ido Azuri[4], Tamar Ziv[5], Reut Hazan Ben-Menachem[6], Shifra Ben-Dor[4], Gil Stelzer[4], Neta Regev-Rudzki[2], Meital Oren-Suissa[11]Brain Sciences, Weizmann Institute of Science, [2]Biomolecular Sciences, Weizmann Institute of Science, [3]Chemical Research Support, Weizmann Institute of Science, [4]Life Sciences Core Facilities, Weizmann Institute of Science, [5]Biology, Technion- Israel Institute of Technology, [6]Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem

Hermaphroditism in *Caenorhabditis* is a latter evolutionary trait, which evolved from the ancestral dioecious (female-male) mating strategy that is common in the group, and is found in *C. afra*. This change in *C. elegans'* mating strategy has impacted the evolutionary forces at play over the species, effectively limiting the role of the males within it, and presumably leading to additional changes in physiology and behavior. However, the ways in which significant changes in the mating strategy of a species affect the behavioral patterns of its individuals, and the underlying sensory sex cues and neuronal outputs have remained mostly unexplored.

We define a new set of previously unknown female mating behavioral features. Strikingly, we found that while *C. elegans* hermaphrodites mostly remain passive or even attempt to escape male mating endeavors, *C. afra* females exhibit great interest in males and actively participate in the mating process, often acting as its initiators. We extracted males-conditioned media and found it mediates a significant and specific attraction of females. In addition, we showed that this attraction is dependent on the release of extracellular vesicles by the males. Analysis of *C. afra* sensory neurons did not reveal any obvious anatomical differences compared with *C. elegans*, suggesting a role for the mating strategy in driving the development of specific functional differences between the two species.

Interestingly, *C. elegans fog-2* sperm-deficient hermaphrodites that were propagated for over 20 generations showed significant interest in mating that was not detected in first-generation *fog-2* animals, and highly resembled that of *C. afra* females. We conducted whole animal RNA profiling and found this newly acquired female mating behavior to be modulated by the downregulation of neuropeptides. Strikingly, global neuropeptides depletion by *egl-3* knock-down as well as knock-down of several specific neuropeptides in first-generation *fog-2* hermaphrodites resulted each in a similar increase in the hermaphrodites' interest in males and sexual behavior.

Our results identified a previously overlooked female mating behavior and reveal some of its underlying mechanisms. Taken together, this points towards the existence of an evolutionary mechanism that could best be described as *sexual adaptation*, whereby the sexual behavior of a species is modified by its mating strategy, shedding some light on the origins of sexual attraction.

72 **The evolution of developmental genetic biases explains the evolution of evolutionary rates** Joao Picao Osorio[1], Charlotte Bouleau[2], Pablo M. Gonzalez de la Rosa[3], Lewis Stevens[3], Nina P Fekonja[4], Mark Blaxter[3], Christian Braendle[2], Marie-Anne P Félix[41]Biology, Institute of Biology of the École Normale Supérieure, CNRS, INSERM, ENS, PSL, [2]Institut de Biologie Valrose, Université Côte d'Azur, CNRS, INSERM, [3]Wellcome Sanger Institute, [4]Institut de Biologie de l'École Normale Supérieure, CNRS, INSERM, ENS, PSL

Random mutation of the genotype does not generate random phenotypic variation because development biases the mutationally inducible phenotypic spectrum. Therefore, understanding such biases in the introduction of phenotypic variation is essential to reveal which phenotypes can be explored and selected in the evolutionary process. Whether such developmental genetic biases in the construction of phenotypic variation influence evolutionary rates is poorly understood.

Here we address this problem by quantifying the relationship between mutation and wild phenotypic variation within and among nematode species. We use the homologous cellular framework of the six vulval precursor cells (VPC), named P3.p to

P8.p, in two clades of nematodes that have divergent evolutionary trajectories of cell fate variation. We generated eight panels of random mutant lines in wild isolates of *Caenorhabditis* and *Oscheius* to quantify the mutability (*i.e.* mutational variance) of VPC fates across micro and macro-evolutionary scales, and compared it with natural genetic variation within and across species of both genera. Our phenotypic analysis of vulva cell fates on over 85,000 nematodes shows a strong alignment of the axes of variation upon random mutation with those of wild variation within each species and genus. When represented in a simplified two-dimensional phenotypic space the direction of mutational and natural variation is along the P3.p axis in *Caenorhabditis*, and along the P4.p axis in *Oscheius*. Interestingly, in both cases, the variable cell fate is sensitive to modulation of the dose of Wnt genes.

Altogether, we show an evolution of the variational properties of VPC fates in *Caenorhabditis* versus *Oscheius*, which can explain the evolution of evolutionary rates.

73 **The co-option of a "grinder-molting protease" is essential for predatory feeding in the nematode *Pristionchus pacificus*** Yuuki Ishita, Takahiro Chihara, Misako OkumuraGraduate School of Integrated Sciences for Life, Hiroshima University

Nematodes exhibit diverse mouth morphologies to adapt to a wide range of food sources including bacteria, plant, and other nematodes. However, the genetic mechanisms underlying the acquisition of novel feeding habits in nematode are largely unknown. While the model nematode *Caenorhabditis elegans* feeds on bacteria using its grinder, the satellite species *Pristionchus pacificus* exhibits novel feeding behavior, predatory feeding on other nematodes with its movable teeth. Previously we showed that serotonin and a subset of serotonin receptors modulate the tooth movement required for opening the prey cuticle; however, evolutionary mechanism of predation remains unclear. Using a forward genetic approach, we found that the astacin metalloprotease *Ppa-nas-6* is required for predation in *P. pacificus*. *Ppa-nas-6* mutants were defective in control of tooth movement during predation and processing of larval cuticle during molting, specifically in the mouth part anterior to the pharynx. In *C. elegans*, *nas-6* is necessary for the molting of the grinder, a feeding apparatus at the posterior part of the pharynx, which is absent in *P. pacificus*. Rescue experiments of *nas-6* in *P. pacificus* and *C. elegans* suggest that alteration of spatial expression patterns rather than the changes in molecular function of *nas-6* could be a key to acquiring the predation-related traits. Reporter analyses with *Ppa-nas-6* promoter in both species suggest the alteration of *nas-6* expression pattern is mediated by *cis* and *trans*-regulatory elements. Our study showed that co-option of a single protease is involved in the evolution of this novel feeding habit.

74 **Development across evolutionary time at a single-cell resolution in the *Caenorhabditis* nematode embryo** Christopher R L Large[1,2], Rupa Khanal[1,2], LaDeana Hillier[3], Chau Huynh[3], Priya Sivaramakrishnan[1], Felicia Peng[1], Qin Zhu[4], Erik Nordgren[2], Jean Rosario[2], Junhyong Kim[2], Robert H Waterston[3], John I Murray[11]Department of Genetics, University of Pennsylvania, [2]Department of Biology, University of Pennsylvania, [3]Department of Genome Sciences, University of Washington, [4]Department of Pharmaceutical Chemistry, University of California, San Francisco

Complex gene regulatory networks specify the development of all multicellular organisms and determine the morphological complexity of life. What determines the rate of change and the evolutionary constraint on cellular gene expression patterns across development remains a fundamental question of biology. Historically, the ability to systematically profile homologous cells across different organisms for their function and transcriptional profile has been limited by technology, however single-cell sequencing has facilitated the ability to capture and molecularly label individual cells through microfluidics. Single-cell sequencing, combined with the defined and evolutionarily conserved developmental lineage of the *Caenorhabditis* nematodes allows for the direct comparison of gene expression between homologous cell-types across evolution. Utilizing these tools, we have measured the spatiotemporal divergence of gene expression across embryogenesis by collecting, annotating and comparing the transcriptomes of homologous embryonic progenitors and terminal cell types using >200,000 and >190,000 single-cells from *C. elegans* and *C. briggsae* respectively. Consistent with the conserved lineage, 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, genes involved with detoxification and response to pathogens, and more surprising, genes including developmental TFs. Comparing cells between species reveals that the neuronal cell types transcriptomes have diverged more than the intestine and body wall muscle. Further work will aim to characterize the driving forces behind the cell-type expression conservation differences and determine the functional consequences of gene expression pattern divergence for developmental regulators through genetic manipulation.

75 **Virus-like transposons cross the species barrier and drive the evolution of genetic incompatibilities** Sonya A. Widen[1], Israel Campo Bes[2], Alevtina Koreshova[1], Pinelopi Pliota[1], Daniel Krogull[1], Alejandro Burga[11]Institute of Molecular Biotechnology, [2]Centre for Genomic Regulation (CRG)

Horizontal gene transfer—the movement of genetic material between species—has been reported across all major eukaryotic

lineages. However, the underlying mechanisms of transfer and their impact on genome evolution are still poorly understood. While studying the evolutionary origin of a selfish element in the nematode *C. briggsae*, we discovered that *Mavericks,* ancient virus-like transposons related to giant viruses and virophages, are one of the long-sought vectors of horizontal gene transfer. *Mavericks*—also known as *Polintons*—are flanked by terminal inverted repeats and can readily jump and insert into genomes, like transposons. But like viruses, they code for a large number of proteins, including a type-B DNA polymerase, a retroviral-like integrase, as well as major and minor capsid proteins. Using a combination of phylogenetics, structural predictions and genetic crosses, we discovered that two novel nematode gene families—*wosp* proteases and *krma* kinases—are preferentially taken up as cargo by *Mavericks* and have been extensively transferred between different nematode species on a global scale. Remarkably, many of these transfers occurred between species that last shared a common ancestor likely hundreds of millions of years ago. We also found that nematode *Mavericks* captured a novel fusogen, MFUS-1, which is remarkably similar in structure to the glycoprotein B from *Herpes simplex virus 1.* This event likely fueled their spread via the formation of enveloped infective particles, analogous to the inception of retroviruses from genomic retroelements. Lastly, we show how the union between a horizontally transferred *wosp* protease, *msft-1*, and a MULE transposon gave birth to a novel class of selfish gene in *C. briggsae*, a mobile toxin-antidote element that causes powerful genetic incompatibilities that drive in wild populations. Our results identify the first wide-spread vector of HGT in animals and highlight how the intertwined biology of viruses and transposons can ultimately impact gene flow between populations, shaping the evolution of the species that carry them.

76 **Nematode-trapping fungus trap *C. elegans* by targeting cuticular collagens** Hanwen Chang, Hung-Che Lin, Ching-Ting Yang, Yen-Ping HsuehInstitute of Molecular Biology, Academia Sinica, Taipei

Cell adhesion is a crucial step in the establishment of infection by pathogens, and it is also the first step in predation by fungal predators on nematode prey. *Arthrobotrys oligospora* is a nematophagous fungus that forms complex adhesive nets to trap nematodes when nutrients are scarce. However, the molecular targets of the adhesion interaction are not well understood. In this study, we conducted forward genetic screens in *C. elegans* to identify mutants that were resistant to *A. oligospora* trapping. We found that loss-of-function mutations in the nuclear hormone receptor *nhr-66*, which acts as a transcription factor, allowed nematodes to escape the trap. Through tissue-specific rescue, we demonstrated that the site of action of NHR-66 is localized in the hypodermis and seam cells. We also performed transcriptomic analysis and found that more than 60 collagen genes were down-regulated in the *nhr-66* mutant. Collagen proteins are major components of the nematode cuticle, and the down-regulation of these genes suggests that altered nematode surface (cuticle) could result in escape behavior. Rescuing the down-regulated collagens in *nhr-66* mutant can abolish the resistant phenotype, revealing that collagens play a crucial role in mediating adhesion between the fungal traps and the nematode prey.

77 **A regulator of nongenetic inheritance mediates the evolution and loss of plasticity** Nicholas A Levis, Erik J RagsdaleBiology, Indiana University

Plasticity is a widespread feature of development, enabling phenotypic change based on the environment. Where such change precedes genetic change, the exploration of alternative phenotypes may promote a trait's evolution, especially when those phenotypes' appearance is followed by plasticity's loss, or genetic assimilation. Although the molecular details of assimilation are largely conjectural, theory suggests a role for epigenetic mechanisms and nongenetic inheritance. Here we show that a regulator of nongenetic inheritance links laboratory evolution of plasticity to cases of assimilation in nature. Using genome-wide evolutionary analyses across nematodes of Diplogastridae, which ancestrally had a polyphenism in their feeding morphology, we found a histone modifier to be under positive selection during both evolutionarily recent and ancient assimilation events. Manipulations of this gene affect both the sensitivity and variation in plastic morphologies, and artificial selection of manipulated lines drive transgenerational shifts in these phenotypes. Our findings point to the plausibility that modified function of this gene sources morphological changes ahead of genetic change, providing an "epigenetic bridge" between an originally inducible phenotype and the assimilation and diversification of new forms. Our results thus give mechanistic insight into how traits are modified as they traverse the continuum of greater to lesser environmental sensitivity.

78 **Hourglass pattern of developmental evolution at the single cell level in *Caenorhabditis elegans*** Fuqiang Ma, Chaogu ZhengThe University of Hong Kong

The phylotranscriptomic analysis of development in several species revealed the expression of older and more conserved genes in midembryonic stages and younger and more divergent genes in early and late embryonic stages, which supported the hourglass mode of development. Whether similar hourglass pattern exists in nematodes and specificaly *C. elegans* is not fully understood. Moreover, previous work mostly studied the transcriptome age of whole embryos or embryonic sublineages, leaving the cellular basis of the hourglass pattern and the variation of transcriptome ages among cell types unexplored. By analyzing both bulk and single-cell transcriptomic data, we studied the transcriptome age of *C. elegans* throughout development. Using the bulk RNA-seq data, we identified the morphogenesis phase in midembryonic development as the phylotypic stage with

the oldest transcriptome and confirmed the results using whole-embryo transcriptome assembled from single-cell RNA-seq data. The variation in transcriptome ages among individual cell types remained small in early and midembryonic development and grew bigger in late embryonic and larval stages as cells and tissues differentiate. Lineages that give rise to certain tissues (e.g., hypodermis and some neurons) but not all recapitulated the hourglass pattern across development at the single-cell transcriptome level. Further analysis of the variation in transcriptome ages among the 128 neuron types in C. elegans nervous system found that a group of chemosensory neurons and their downstream interneurons expressed very young transcriptomes and may contribute to adaptation in recent evolution. Finally, the variation in transcriptome age among the neuron types, as well as the age of their cell fate regulators, led us to hypothesize the evolutionary history of some neuron types.

79 **A toxin-antidote element in *Caenorhabditis elegans* that causes L1 larval arrest** Laura Walter-McNeill, Stefan Zdraljevic, Heriberto Marquez, Leonid KruglyakUniversity of California, Los Angeles

Toxin-antidote (TA) elements are selfish genetic elements which increase their own frequency by killing non-carrier progeny. To search for novel TA elements in *C. elegans*, we crossed two highly divergent wild strains isolated from Hawaii (HI) and California (CA). We genotyped the progeny over several generations and observed a strong depletion of CA alleles on the right arm of chromosome V (chrVR) after only four generations, suggesting the presence of a TA element on chrVR. In self-crosses of CA/HI hermaphrodites, 25% of the progeny arrested at the L1 larval stage. All arrested individuals were homozygous for the CA genotype at chrVR. When we crossed CA/HI hermaphrodites to CA males, 50% of the resulting progeny arrested as L1s, while the reciprocal cross did not produce any arrested progeny. Based on this inheritance pattern, we hypothesized that a maternally inherited toxin is present in the HI strain.

We created a near-isogenic line with a region on chrVR introgressed from HI into CA. This line recapitulated the lethality phenotype, confirming that it contained the TA element. Further localization of the element was limited by low recombination rates in the region. To overcome this, we developed a technique to induce targeted recombination with Cas9, which enabled us to quickly resolve the element to 10 genes. A knockout of one gene in HI led to loss of lethality in crosses to the susceptible strain, showing that this gene (htox-1; HI toxin) encodes the toxin. A knockout of another gene could not be made homozygous unless htox-1 was first deleted, identifying this gene (hant-1; HI antidote) as the antidote. We show that hant-1 is sufficient to prevent htox-1 lethality using an hant-1 rescue plasmid. We are using fluorescently tagged and inducible versions of htox-1 and hant-1 to determine where and when these genes function.

The HI TA haplotype is present in 29 highly divergent *C. elegans* strains (5.3% of 550 CeNDR strains), which are found exclusively on the Hawaiian islands. All but four of these strains were collected in the Kokee State Park, and only three *C. elegans* isolates from this locality do not contain the TA element. This suggests that the HI TA element has nearly fixed in this subpopulation. One hypothesis is that *C. elegans* evolved in the Pacific Islands; if this is indeed the case, the HI TA haplotype may represent the ancestral state of the species, with the element becoming lost as the ecological range of *C. elegans* expanded.

80 **The growth rate of *C. elegans* is modulated by the *Actinobacteria* in its microbiome via sulfur metabolism** Om Patange[1], Peter Breen[2], Gary Ruvkun[11]Genetics/Molecular Biology, Harvard Medical School/Massachusetts General Hospital, [2]Cell Biology and Physiology, School of Medicine, University of North Carolina

Weak interactions between animals and microbiome are the most abundant interactions, but difficult to study. Past work has often been focused on strong pathogenic and commensal relations with facile phenotypes. Here, we uncover the molecular basis of a pervasive weak interaction. By growing *C. elegans* on a panel of phylogenetically diverse microbiome species one at a time, we found that *C. elegans* grown on bacteria of the *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* clades have sublethal developmental delays. *C. elegans* grow up to 2x slower on these bacteria compared to growth on *E. coli* and most other *Proteobacteria*. A forward genetic screen of *C. elegans* alleviating this delay on an *Actinobacteria* species, *Microbacterium* sp., revealed that key genes of the sulfur metabolism pathway, *cth-2* (CystaTHionine gamma lyase) that produces cysteine and *mars-1* (Methionyl Amino-acyl tRNA Synthetase), are part of the slow growth program. Loss of *cth-2* causes larval arrest of animals, opposite to the effect observed with the hypomorph alleles isolated in the screen. A second forward genetic screen for rescue of *cth-2 (null)* larval arrest on *Microbacterium*, further implicated cysteine metabolism via the gene *cars-1* (Cysteinyl Amino-acyl tRNA Synthetase). From the first screen, we also isolated an uncharacterized Leucine-Rich Repeat containing protein, here named *lrr-2*. We showed with epistasis experiments that *lrr-2* acts via *cdo-1* (Cysteine DiOxygenase), a cysteine degrading protein that acts downstream of *cth-2*, implicating *lrr-2* in the regulation of cysteine levels. Animals grown on *Microbacterium* exhibit a lipid droplet accumulation phenotype, which is similar to a *sams-1* and *pmt-1* knock-down phenotype observed in literature. Exogenously supplied metabolites along the sulfur metabolism pathway to *C. elegans* mutants grown on *Microbacterium* showed that exogenous cysteine but not methionine rescued *cth-2* null animals and that methionine, vitamin B12, and choline rescued *WT* animals. Taken together, and combined with the classical methionine sparing effect of cysteine, these findings implicate phosphatidylcholine production inhibition as the cause for growth retardation on *Microbacterium*. Testing the *C. elegans* mutants on the microbiome collection revealed that this sulfur metabolism pathway rescues growth retardation by *Actinobacteria*, but not *Fir-*

micutes nor *Bacteroidetes*. Our work gives mechanistic insight on animal-microbial interactions mediated by sulfur metabolism.

81 **Variable recombination rate landscapes and adaptation to a novel environment** Tom Parée, Henrique TeotonioEcole Normale Superieure

Theory suggests that by reducing selective interference recombination increases adaptive rates. We use experimental evolution in *Caenorhabditis elegans* populations with alternative modifiers of the recombination rate landscape to test for adaptation. *C. elegans* exhibits a wild-type recombination landscape with low recombination rates in chromosomal centers and high recombination rates chromosomal arms. Loss of function of the *rec-1* gene equalizes recombination rates between centers and arms, without changes in the total recombination map length. In our populations, most fitness loci are located in the chromosomal arms. We challenged populations with standing genetic variation but different *rec-1* alleles (wild-type vs. loss-of-function mutant) to a novel environment. After 40 generations of evolution, we measured a fitness-proxy and, during the experiment, patterns of genome-wide SNP allele frequency change. These data indicate that the wild-type *rec-1* allele increases adaptive rates by decreasing the extent selection interference, thus confirming theoretical expectations.

82 **Natural polymorphism in biofilm-mediated killing of adult *C. elegans* involves surface galactans** Jonathan HodgkinBiochemistry, Univ Oxford

The bacterial pathogen *Yersinia pseudotuberculosis* (YPIII) can cause mild disease in humans and may lead to lethal infection in many mammals and birds. When growing on YPIII lawns, larvae of *C. elegans* accumulate bacterial biofilms on their heads, which impair growth. All tested natural races of *C. elegans* accumulate non-lethal Yp biofilms as L1 larvae. In addition, adults of the Bristol strain N2 and a minority (6/35) of natural isolates are rapidly killed by Yp biofilm, as a result of polymorphism at one major locus, termed *yaks-1*(Yersinia Adult Killing Sensitivity). Sensitive strains have been found in England, France, Spain, Madagascar, Australia and California. The natural allele *yaks-1(e3150)* introgressed into N2 (Bristol) from CB4856 (Hawaii) confers resistance to adult killing. Genetic mapping has narrowed the location of *yaks-1* to a 40 kb interval on LGI, which contains no obvious candidate genes. Mutants in *bah* (Biofilm Absent on Head) genes, which were originally discovered by Creg Darby, are resistant to both larval and adult Yp biofilm and grow well on YPIII lawns. The genes *bah-1, bah-2* and *bah-4* all encode predicted galactan synthases (GT92 family). Down-regulation of such genes in adult worms could explain resistance to killing. Susceptibility to larval biofilm formation can be transferred to resistant larvae by direct contact between worms. The transferable agent is insoluble, resistant to proteolysis and may be a complex galactan.

83 **Nutritional programming of host-microbiome interactions in Caenorhabditis elegans** Dana Blackburn, Adrien Assié, Daniela Vidal Vilchis, Buck S SamuelAlkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine

All organisms need amino acids (AAs) to survive and yet only a handful can synthesize all of them de novo, resulting in collaboration and competition among organisms. To chart the role of AAs in regulating host-microbe interactions, we disrupted the flow of AAs at different critical points in a system composed of Caenorhabditis elegans and a two-member microbiome with Ochrobactrum, a metabolically versatile and efficient gut colonizer, and Myroides, a more limited bacterium.

Using BiomeProfiler, a pipeline that can predict microbial community metabolic and functional profiles, we identified several AA metabolic pathways driving community composition in a nutrient-poor environment. To test the role of these AAs in microbiome assembly, we disrupted the availability of these AAs in the environment by removing one at a time. Furthermore, C. elegans can both utilize and contribute AAs within the host-microbe system. We examined the impact of the host's AA contributions by disrupting host AA transporters (AAT) via RNAi knockdown. Finally, to identify the effects of bacterial AA production on gut composition, we tested colonization with Myroides with either of two Ochrobactrum mutants, a valine/isoleucine (VAL/ILE) mutant, and a threonine (THR) mutant.

AA disruption at all levels resulted in community composition shifts to varying degrees. Most notably, the inability of Ochrobactrum to produce VAL/ILE or THR shifted the balance to an increase in Myroides suggesting that Myroides, which is unable to naturally produce VAL/ILE or THR, is a better AA scavenger in nutrient-limited conditions. However, the Ochrobactrum-Myroides balance was restored when we increased gut levels of VAL and THR by inhibiting the host's ability to uptake valine or threonine via host insulin-signaling pathways. These results show the delicate balance between AA from the environment, host, and microbes with VAL and THR being two critical AA, while ILE has less of an effect of the microbiome balance.

Our work serves as a foundation for identifying the molecular networks involved in the nutritional programming of host-microbiome interactions, which could aid in developing new strategies to promote gut health and/or prevent pathogen colonization.

84 **The molecular atlas of adult *C. elegans* glia across sexes reveals sexually dimorphic and heterogenous glia** Maria D Purice, Elgene JA Quitevis, R. Sean Manning, Liza J Severs, Nina-Tuyen D Tran, Violet Sorrentino, Manu D Setty, Aakanksha D

SinghviFred Hutchinson Cancer Research Center

A comprehensive description of nervous system function, and sex dimorphism within, is incomplete without clear assessment of the diversity of its component cell types, neurons and glia. *C. elegans* has an invariant nervous system with the first mapped connectome of a multicellular organism. Here we present single nuclear RNAseq evaluation of glia across the entire nervous system of adult *C. elegans* in both sexes, complementing previous single-cell analysis of its neurons. Our data identify both sex-shared and sex-specific glia, and iterative computational and machine learning models reveal glial subclasses. We have identified glia-specific, subclass-specific and pan-glia molecular markers, some of which we have validated *in vivo* using transcriptional reporters. Comparative analysis also reveals previously unappreciated molecular dimorphism in anatomically identical glia between and within sexes, indicating functional heterogeneity between these. Furthermore, gene ontology comparisons between tissue types reveals that adult *C. elegans* glia express neuropeptide genes, but lack the canonical *unc-31/CAPS* dependent dense core vesicle release machinery, implying that glia deploy an alternate neuromodulator processing mechanism. Overall, this molecular atlas of adult glia reveals detailed insights into glial heterogeneity and sex dimorphism in the adult C. elegans nervous system, and is available as a searchable three-dimensional atlas at www.wormglia.org.

85 **Loss of poly(U) polymerases has global impacts on the small RNAome and disrupts early embryo PGL granule clearance** Leanne H Kelley, Ian V Caldas, Yini Li, Ashley Houlihan, Matthew Sullenberger, Yasir Ahmed-Braimah, Eleanor M MaineBiology, Syracuse University

Uridylation, the addition of uridine to the 3' end of RNAs, is a conserved modification linked to RNA turnover, including maternal mRNA clearance, during early embryogenesis in many organisms. Poly(U) polymerases are responsible for this 3' tailing modification in *C. elegans*. Our previous work has shown that PUP activity is critical for germline development and embryonic viability, especially under temperature stress. Having characterized the phenotypes of *pup-1*, *pup-2*, *pup-3*, and *pup-4* single, double, triple, and quadruple null mutants, our current goal is to identify PUP RNA targets and determine how their uridylation alters RNA expression to coordinate proper germline development.

To obtain a comprehensive picture of uridylation in the small RNAome and transcriptome, we carried out RNA-seq experiments. The proportion of U-tailed siRNAs targeting any given gene ranges wildly from 0-79%. MiRNAs and piRNAs are rarely U-tailed. SiRNA U-tailing is very reduced in any strain carrying *pup-1*, with the largest reduction in *pup-1/-2* double mutants. Globally, increased siRNA expression correlates with reduced U-tailing, suggesting U-tails limit siRNA stability; however, some siRNAs decrease in expression despite U-tail loss, suggesting an additional function. To add to uridylation's complex functional significance, ALG-3/-4-associated siRNAs are elevated in *pup-1/-2* mutants but are reduced in *pup-1* and *pup-3;pup-1/-2* mutants, which may contribute to the more severe defects in *pup-1/-2* compared to the other two strains. Preliminary Nanopore sequencing data show that U-tailing occurs on mRNAs but reveal no significant differences in frequency between wildtype and *pup* mutants.

Consistent with severe germline and embryonic defects, Illumina mRNA-seq identified altered expression of many germline-expressed mRNAs in *pup* mutants. For example, consistent with reduced expression of autophagy genes, we found that *pup-1* and *pup-1/-2* mutant embryos at 25°C do not clear somatic PGL granules. Hence, *pup-1* is seemingly not required for PGL granule formation but may be essential for their clearance. Strikingly, these mutants also have delayed primordial germ cell (PGC) division. At embryonic stages when there should be two PGCs, some *pup-1* and most *pup-1/-2* embryos have only one PGC as identified by PGL-1 perinuclear foci. Preliminary data suggest that the delayed PGC divisions contribute to reduced fecundity in adults. In addition, rare F2 embryos have no PGL-1-marked PGC, suggesting failure to specify a germline; this phenotype is more penetrant in F3 embryos. Tying in the fact that mRNAs encoding a few major RNAi players are reduced in early embryos, PUPs appear to influence both sRNAs and mRNAs, whether directly or indirectly, to aid in germline establishment in embryos and adults.

86 **A mechanistic link between histone mRNA homeostasis and piRNA biogenesis** Joana Pereirinha[1], Anke Busch[1], Ann-Sophie Seistrup[1], Nadezda Podvalnaya[1], Ricardo Cordeiro Rodrigues[1], Kamila Delaney[2], Florian Steiner[2], Julian König[1], René Ketting[11]Institute of Molecular Biology, Mainz, [2]Dept. of Molecular and Cellular Biology, University of Geneva

The piRNA pathway is a highly conserved small RNA pathway, best known for its function in silencing transposable elements (TEs) and in germline development. In *C. elegans*, piRNAs (21U-RNAs) target mostly germline mRNAs and their effect in TEs regulation is modest, and in contrast to other species, the loss of the piRNA pathway does not lead to acute sterility. The PETISCO complex was found to be required for 21U-RNA production. Even though 21U-RNAs are dispensable for development, PETISCO is required for embryonic development: mutants show a maternal effect lethality (Mel) phenotype. These two functions depend on two different PETISCO-binding proteins, PID-1 and TOST-1, respectively. I aim to understand the function of PETISCO during embryogenesis.

Using iCLIP against the PETISCO subunit TOFU-6 we found that PETISCO binds to the 3'-terminal regions of all four replication-dependent histone (RDH) mRNAs. This interaction may be dependent on specific features of histone mRNAs, such as the absence of splicing and the presence of a stem-loop structure. We also found that in *tost-1* mutants, RDH mRNAs were severely downregulated, and deleting a histone cluster enhanced the Mel phenotype of a hypomorphic *tost-1* mutant. These data suggest that the Mel phenotype of PETISCO mutants may be linked to defects in RDH mRNA homeostasis in early embryos. This hypothesis is consistent with the fact that we find premature activation of genes during embryogenesis, a process that is affected by the concentration of maternally deposited RDH mRNAs. To test this idea further, we asked whether stabilizing RDH transcripts might rescue the Mel phenotype of PETISCO mutants. Indeed, we observed that mutants in the RDH mRNA degradation pathway, can partially rescue the Mel phenotype. We conclude that maternally deposited PETISCO:TOST-1 likely stabilizes maternal RDH mRNAs, which in turn are essential during embryogenesis.

Evolutionary analysis of PID-1 and TOST-1 indicates that the RDH mRNA stabilization function likely pre-dates PETISCO's role in 21U-RNA production. Hence, 21U-RNA biogenesis may have evolved from a maternal mRNA stabilization mechanism. The common logic between these processes may be that PETISCO stabilizes transcripts that are inherently unstable for use in downstream processes. For the PETISCO:PID-1 this would be 21U-RNA precursor processing (see abstract by Podvalnaya et al.), while for PETISCO:TOST-1 this would be translation of histones during embryogenesis.

87 **Uncovering the hidden germline genome: multimegabase tandem repeats and eliminated DNA in *Auanema rhodensis*** Pablo Gonzalez de la Rosa[1], Liesl G. Strand[2], Sally Adams[3], Margrethe Johansen[4], Manuela Kieninger[1], Anne M. Villeneuve[2], Andre Pires-daSilva[3], Mark L. Blaxter[11]Tree of Life, Wellcome Sanger Institute, [2]Departments of Developmental Biology and Genetics, Stanford University, [3]School of Life Sciences, University of Warwick, [4]University of Nottingham

Auanema rhodensis is a free-living nematode species that exhibits a non-Mendelian X chromosome segregation. Specifically, hermaphrodite sperm in *A. rhodensis* always contain two copies of the X chromosome, while oocytes contain no X chromosomes. Previously it was established that the 60 Mb *A. rhodensis* genome consists of 7 chromosomes, based on a genetic linkage map and microscopy. However, until now, there were no reports of chromatin diminution in *Auanema* nematodes. Here we report that approximately 95 Mb (60%) of the *A. rhodensis* genome are eliminated from somatic cells during development, including a large number of tRNAs, rRNAs, and hundreds of protein coding genes that were previously undetected. We found that multimegabase tandem repeats make up the majority of the eliminated DNA, which we validated by PCR and FISH. Furthermore, our genome assembly showed that all chromosomes have exactly one eliminated region at its centre, which in the autosomes contain large tandem repeats. In addition, all chromosomes possess large tandem repeats at each termini. While these sequences are similar between termini of autosomes, they are different from those of the X chromosome. Our findings suggest that the eliminated DNA in *A. rhodensis* serves different functions: germline maintenance and centromeric. A centromeric function could help explain the non-Mendelian segregation of the X. Altogether, our findings shed light on the complex and dynamic nature of nematode genome evolution, and highlight the importance of carefully examining repetitive regions and genomic elimination processes. Further studies on the role and function of these multimegabase tandem repeats in *A. rhodensis* could provide valuable insights into the unorthodox patterns of meiotic X chromosome segregation and the function of the large tandem repeats that are commonly removed from the soma in animal species undergoing programmed DNA elimination.

88 **TASOR triggers intron-less gene silencing in *C. elegans*** Yekaterina V Makeyeva[1], Min Li[1], Takao Ishidate[2], Masaki Shirayama[2], Craig C Mello[21]RTI, UMass Chan Medical School, [2]UMass Chan Medical School

We have previously shown that silencing of intron-less genes is initiated independently of the piRNA pathway but activates the same downstream WAGO 22G-RNA-dependent transgenerational silencing pathway (1). However, how intron-less genes are channeled into the WAGO 22G-RNA pathway remains unknown.

Here we show that a worm ortholog of *Tasor* (*tsr-1*), a component of the human silencing hub (HUSH) complex, is required for initiation of the intron-less gene silencing. The HUSH complex binds to and represses a subset of human intron-less genes, including an autonomous mobile retrotransposon LINE-1, by promoting *MORC2* (*morc-1* in *C. elegans*) and *SETDB1* (*met-2* in *C. elegans*)-mediated heterochromatin modifications (2, 3). We found that mutating *tsr-1* in animals containing a previously silenced intron-less reporter failed to de-silence the reporter. While, in contrast, mutating *tsr-1* prior to the introduction of the same intron-less reporter resulted in reporter expression. Our findings suggest that TSR-1 and other components of the *C. elegans* HUSH complex establish heterochromatin on genes with overly long exons. Transcription within the TSR-1-induced heterochromatin may generate templates that are transcribed by RNA-dependent RNA Polymerase (RdRP) to produce WAGO 22G guide RNAs that program WAGO-clade Argonautes.

Our findings reveal the existence of an evolutionally conserved genome-surveillance system that installs heterochromatin on intron-less genes, and identify a third mechanism, in addition to dsRNAs and piRNAs, that can induce transgenerational silencing

in *C. elegans*. Interestingly, in this case, the initiating cue appears to be the quality of pre-mRNA processing in the nucleus.

(1) Makeyeva *et al*., *Dev. Cell* **56**, 2636 (2021)

(2) Liu *et al.*, *Nature* **553**, 228 (2018)

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89 **3D chromosome organization in *Caenorhabditis elegans* autosomes.** Dania Camila Pulido Barrera, Ahilya N Sawh, Susan E MangoBiozentrum, University of Basel

Topologically Associating Domains (TADs) are a conserved feature of chromosome organization in which contiguous sequences form a folded domain that is bounded and separated from adjacent DNA. *C. elegans* possesses large-scale TADs on the X chromosome and smaller, less insulated TADs on autosomes. We have used single-molecule image analysis (chromosome tracing) to determine if *C. elegans* possesses megabase (Mb) TADs that resemble those of other animals including mammals. Our results show that autosomes are organized into TAD-like structures. These TAD-like domains possess strong boundaries and have the same genetic requirements as mammalian TADs, distinguishing them from another organizational structure called a compartment. Our work brings *C. elegans* chromosome organization in line with other species and reveals the folding principles of chromosomes at Mb-scale.

90 **The functional cooperation of conserved RNA-binding proteins ensures the silencing of a master regulator** Daria Sobanska[1], Alicja A Komur[1], Agnieszka Chabowska-Kita[1], Julita Gumna[1], Pooja Kumari[2], Katarzyna Pachulska-Wieczorek[1], Rafal Ciosk[21]Institute of Bioorganic Chemistry, PAS, [2]University of Oslo

Regnase-1 (Zc3h12a or MCPIP1) is an evolutionarily conserved endoribonuclease that degrades specific mRNAs involved in many essential biological processes including immune homeostasis, development, and cancer. It was initially reported to cooperate with another RNA-binding protein (RBP), Roquin-1, which was suggested to recruit Regnase-1 to specific mRNA targets during T-cell activation [1]. This model was additionally supported by a more recent study showing that a physical interaction between these two RBPs is crucial for mRNA silencing [2]. However, studies performed in different cell types led to an alternative model, postulating that Regnase-1 and Roquin-1 regulate mRNAs independently from each other and through entirely different mechanisms [3]. According to these studies, Regnase-1 cooperates with Upf1 helicase that unwinds the mRNA targets, while Roquin-1 promotes exonucleolytic degradation of transcripts by the interaction with the CCR4-NOT deadenylase complex.

Studying the *C. elegans* ortholog of Regnase-1, called REGE-1 (REGnasE-1), we have uncovered its functional cooperation with the nematode counterpart of Roquin-1, RLE-1. Although REGE-1 and RLE-1 associate with mRNA independently, both proteins are essential for mRNA silencing. In contrast to mRNA regulation by mammalian protein, REGE-1-mediated mRNA silencing in *C. elegans* functions independently from the nematode counterpart of Upf1, SMG-2, mRNA deadenylases, and decapping enzymes. Interestingly, we found that other protein partners of REGE-1 might also be involved in mRNA regulation. Thus, the requirement of protein co-factors seems to exhibit species-specific variation. Moreover, in contrast to Regnase-1, which regulates a set of diverse transcripts, REGE-1 targets a single mRNA encoding a conserved transcription factor, ETS-4. Thus REGE-1, by controlling the abundance of ETS-4, affects the transcription of diverse downstream genes regulating various aspects of animal physiology. Collectively, our studies suggest that although REGE-1/Regnase-1 are functionally related from nematodes to humans, the exact mechanisms underlying the mRNA regulation vary between species.

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91 **Biotinylation-based tissue-specific ChIP-Seq reveals context-dependent genomic distribution of histone variants H2A.Z and H3.3** Idris Selman Bulut[1], Gizem Köse[1], Marcel Studt[1], Sevinc Ercan[2], Baris Tursun[11]Molecular Cell Biology, Institute of Cell and Systems Biology of Animals, University of Hamburg, [2]New York University

Genomic occupancy of chromatin factors is important for spatial and temporal gene expression control, which is crucial during biological processes including cell fate specification and development. Many chromatin factors have pleiotropic functions and are often expressed in several tissues. Analyzing their tissue-specific genome occupancy by chromatin immunoprecipitation with sequencing (ChIP-Seq) allows for dissecting gene expression regulatory programs driving cell type-specific processes. The

two conserved histone variants H2A.Z and H3.3 are chromatin components, which presumably have context-dependent effects on gene regulation during differentiation, function, and maintenance of specific cell types. Yet, it remained unknown whether genomic loci are occupied by these histone variants in a tissue-specific manner. We applied an *in vivo*biotinylation-based tissue-specific chromatin immunoprecipitation (ChIP-Seq) procedure, which we termed Bio-ChIP, to assess the genomic distribution of H2A.Z and H3.3 in specific tissues of the worm. Bio-ChIP does not require cell sorting or overexpression of the target protein as it makes use of CRISPR/Cas9- mediated addition of a biotinylation recognition sequence and tissue-specific co-expression of the biotin ligase BirA.

Applying Bio-ChIP to assess the genomic distribution of H2A.Z and H3.3 specifically in neurons, intestine, muscle, and the germline yields high-quality ChIP-Seq results, which are superior to standard antibody-based procedures that reflect mixed tissues and provide limited insight. Our Bio-ChIP results demonstrate that H2A.Z displays similar genomic distribution in the compared tissues with a subset of genomic loci that are occupied in a tissue-specific manner. In contrast, H3.3 has DNA-binding profiles with high specificity in different tissues. Among others, our results revealed tissue-specific occupancy of numerous piRNAs loci by H3.3 in the soma such as neurons, where also genomic loci harboring other species of small RNA display H3.3 occupancy. Furthermore, we applied Bio-ChIP during aging and can observe high H3.3 dynamics in the intestinal chromatin, suggesting a correlation between H3.3 displacement and aging.

Overall, Bio-ChIP of histone variants and other chromatin factors can reveal tissue-specific chromatin regulation and gene expression modes that were previously masked by data derived from mixed tissues.

92 **A family of F-box/transposase fusion genes involved in germ cell proteostasis and thermotolerance** Miguel V. Almeida[1], Zixin Li[2], Alexandra Dallaire[1], Lukas Fiedler[1], Xiaodan Liu[1], Falk Butter[3], Eric A. Miska[4], Christian Rödelsperger[21]Department of Biochemistry, University of Cambridge, [2]Department for Integrative Evolutionary Biology, Max Planck Institute for Developmental Biology, [3]Institute of Molecular Biology, [4]University of Cambridge

Transposable elements (TEs) can be co-opted for novel biological functions. One evolutionary path to TE co-option is the capture of TE-derived protein-coding sequences by an endogenous gene, resulting in fusion proteins that may evolve new gene regulatory functions. We identified a *Mariner* transposase helix-turn-helix (HTH) DNA-binding domain that was captured in the *Caenorhabditis* genus by a subset of F-box genes, which we refer to as *fbxa-hth* genes. The origin of *fbxa-hth* genes likely occurred through a single capture event in the ancestor of the Elegans group, followed by an increase in copy number. We focused on *fbxa-215*, a *fbxa-hth* gene which is highly expressed in the germline and embryos and localizes to germ granules in embryos. In-frame deletion of the HTH domain of *fbxa-215* uncovered a fully penetrant egg-laying defect, highlighting the importance of this domain. In fact, while their F-box domains are evolving rapidly, the HTH domains of *fbxa-hth* genes display signatures of purifying selection, indicating functional constraints. Quantitative proteomics shows interactions between FBXA-215 and factors associated with E3 ubiquitin ligase complexes, proteasome, and stress response, suggesting a function in proteostasis, consistent with previously described roles of other F-box proteins. The TE-derived HTH domain of FBXA-215 interacts with proteins required for translation and stress response, indicating that the HTH domain was repurposed to bind additional protein substrates. We observe TE enrichment in proximity to F-box genes in the *Caenorhabditis* genus, suggesting that abundant neighbouring TEs may have provided opportunity for the HTH capture and facilitated subsequent integration of *fbxa-hth* genes into existing cis-regulatory networks. *fbxa-215* has upstream Helitron TEs that modulate its expression upon heat-shock, via Heat-shock factor 1 (HSF-1). Consistent with integration in a heat-shock network, *fbxa-215* mutants display higher tolerance to elevated temperature than wild-type animals. We found and validated additional instances of TE and viral sequences captured by F-box genes across the eukaryotic tree-of-life, predominantly in plants. Overall, these findings demonstrate recurring TE/F-box gene fusions that potentially fuel novel forms of gene regulation in eukaryotes.

93 **Incorporation of multiple non-canonical amino acids using genetic code expansion in *C. elegans*** jose J vazquez[1], Sebastian Greiss[21]Center for Discovery Brain Sciences, University of Edinburgh, [2]University of Edinburgh

Expanding the genetic code allows for the co-translational incorporation of amino acids beyond the canonical 20 found in nature. Such additional non-canonical amino acids (ncAAs) can impart new functionalities to existing proteins. Examples of ncAA currently available for use include, among others, photo-caged amino acids, which allow for the design of light-activatable proteins, and amino acids carrying bioorthogonal chemical handles, which can be used to site-specifically attach labels or other functional groups to proteins.

In its most simple form, genetic code expansion to incorporate ncAA into proteins requires an orthogonal aminoacyl tRNA synthetase (aaRS) /tRNA pair to be introduced into the host organism. The orthogonal aaRS must specifically recognize a ncAA and use this amino acid to specifically aminoacylate its cognate orthogonal tRNA, which is itself not a substrate for endogenous synthetases. The aminoacylated tRNA then decodes a designated codon not assigned to another amino acid, usually a UAG (amber)

stop codon, introduced into a gene of interest at a specific site.

We have previously established efficient ncAA incorporation in *C. elegans* using either a UAG or a UAGA quadruplet codon to specify the incorporation site. We have used the approach in *C. elegans* to express photo-activatable versions of Cre recombinase for optical control of gene expression, and of human Caspase-3 for optical control of cell ablation[1, 2].

We have now further developed this technology to allow independent incorporation of two different ncAAs at the same time. For this, we established mutually orthogonal aaRS/tRNA pairs in worms, and we engineered tRNA variants to allow decoding of different triplet and quadruplet codons.

With this system for independently incorporating different ncAAs we can, for example, optically control both FLP and Cre recombinases in the same animal. We are developing a method that will allow the use of photo-activateable Cre and FLP to independently switch on or off expression of two or more target genes. By employing a microscope mounted laser such control will be possible with single cell precision.

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[2] Xi et al. “Using a quadruplet codon to expand the genetic code of an animal”. In: Nucleic acids research 50.9 (2022), pp. 4801–4812.

94 **Global analysis of RNA-binding protein expression and subcellular localization in *C. elegans*** John Laver[1], Maida Duncan[1], Mihail Sarov[2], John Calarco[11]Department of Cell and Systems Biology, University of Toronto, [2]Max Planck Institute of Molecular Cell Biology and Genetics

The regulation of post-transcriptional processes, including mRNA splicing, localization, translation, and stability, plays an essential role in the control of gene expression. These processes are regulated by *trans*-acting factors, such as RNA-binding proteins (RBPs) and small RNAs. To better understand the systems-level organization of post-transcriptional regulation in a multicellular organism, we are undertaking a global analysis of the expression and subcellular localization of RBPs in *C. elegans*. We have compiled a comprehensive list of *C. elegans* RNA-binding and regulatory proteins, comprising 284 proteins containing sequence-specific RNA-binding domains, 142 proteins containing other domains with roles in RNA metabolism, 895 proteins with functional annotations related to RNA regulation, and 803 proteins identified in previously published RNA interactome capture studies. We analyzed the expression of these proteins across tissues and development using published RNA-seq data, which revealed that most are expressed predominantly in a single tissue. The majority are expressed most highly in the gonad, which may reflect the importance of post-transcriptional regulation in the development of the germline. Among somatic tissues, the intestine and neurons had the highest number of tissue-enriched RBPs, and there were several RBPs enriched in each tissue across development, highlighting their potential for sculpting tissue-specific gene expression patterns. Interestingly, RBPs with more tissue-specificity in expression are less likely to have direct orthologs in other organisms. To examine RBP expression and subcellular localization patterns at the protein level, we are using a microscopy-based approach, with a particular focus on the expression of sequence-specific RBPs in neurons. To date, we have examined 53 GFP-tagged RBPs, revealing a diversity of expression patterns. Within the nervous system, half of the RBPs are expressed in only a subset of neurons. Approximately equal proportions localize to either the cytoplasm, the nucleus, or both, with a variety of different localization patterns observed in each compartment. Intriguingly, several RBPs display different subcellular localization patterns in different neurons. Taken together, this work will provide a detailed understanding of where and when each RBP is expressed, thus yielding important insights into RBP regulatory networks controlling tissue-specific development and function.

95 **High-Throughput Library Transgenesis in *Caenorhabditis elegans* via Transgenic Arrays Resulting in Diversity of Integrated Sequences (TARDIS)** Zachary C Stevenson[1], Megan J Moerdyk-Schauwecker[1], Stephen A Banse[1], Dhaval S Patel[2,3,4], Hang Lu[2,3], Patrick C Phillips[11]Biology, University of Oregon, Institute of Ecology and Evolution, [2]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, [3]Petit Institute for Bioengineering and Bioscience, [4]NemaLife

DNA libraries are collections of sequences that can be used to systematically explore genetic features and functions including, protein variants, protein-protein interactions, promoter elements, barcodes, guide RNA libraries, and many more applications. Libraries can be relatively simply introduced into single cells using standard transformation techniques. However, most methods for introducing these libraries into multicellular organisms rely on microinjection techniques that are inefficient and labor-intensive. Therefore, there is a need for a simple yet powerful approach to large-scale transgenesis using DNA libraries in multicellular organisms.

We developed a new method called Transgenic Arrays Resulting in Diversity of Integrated Sequences (TARDIS) that can create

thousands of transgenic individuals with different library sequences in multicellular systems. TARDIS works by first inserting a large library cassette, the TARDIS library array (TLA) into a single individual using standard methods. This library cassette contains diverse DNA elements flanked by homology arms for integration into a synthetic landing pad engineered within the genome. Heat shock activation of Cas9 then targets the synthetic landing pad and integrates a sequence at random from the TLA, which is stably inherited by the offspring. Thus, transformation of a single individual with a TLA followed by lineage expansion and induced transgenesis can give rise to thousands of genetically unique transgenic individuals.

We used TARDIS in Caenorhabditis elegans to generate two types of transgenic arrays: (1) unique, random barcodes and (2) promoters from a pre-defined library. For the first type, we showed that TLAs can contain several thousand unique barcode sequences. The associated landing pad is designed to allow random barcode integration within a synthetic intron leading to Hygromycin B resistance only upon integration. For the second type, we generated TLAs containing promoter sequences driving either mScarlet-I or mNeonGreen expression upon integration into specialized landing pads. By utilizing a delayed-integration approach, we found that TARDIS increased transgenesis efficiency by up to ~1000 times compared to existing methods that rely on single-step integration.

96 **Towards Spatial Transcriptomics of C. elegans via Expansion Sequencing** Ruihan Zhang[1,2], Chi Zhang[1,2], Yangning Lu[1,2], Emma Besier[3], Mansour Alawi[4], Yves Quéméner[4], Yosuke Bando[1,5], Madison A. Sneve[1], Donglai Wei[6], Edward S. Boyden[1,2], Brett Pryor[11]Massachusetts Institute of Technology, [2]Howard Hughes Medical Institute, [3]Harvard University, [4]Fixstars Solutions, Inc., [5]Kioxia Corporation, [6]Boston College

Spatial transcriptomics, the analysis of gene expression in the context of cellular and tissue organization, has emerged as a powerful tool in functional genomics. Spatial transcriptomics of *C. elegans* can facilitate the profiling of cell states and gene expression throughout the entire animal. However, the small size of *C. elegans* and its cells presents a challenge that requires nanoscale spatial resolution for spatial transcriptomic analysis.

Expansion sequencing (ExSeq) is a spatial transcriptomics technique that performs in situ sequencing of physically expanded specimens, allowing for multiplexed mapping of RNAs at nanoscale, subcellular resolution throughout intact tissues. This study aimed to explore the potential of ExSeq for spatial transcriptomics of *C. elegans*.

To enable spatial transcriptomics of *C. elegans*, we optimized the ExSeq pipeline to be compatible with different developmental stages. We first validated the protocol with a ten gene set of tissue and cell type marker genes including various tissue markers and neuronal cell type markers. Our results showed that ExSeq revealed the expected tissue and cellular locations of these genes.

After validating the protocol for analyzing gene expression throughout the entire animal as described above, we focused on a deeper analysis of the transcriptomic landscape of the nervous system of *C. elegans*. Specifically, we aimed to map known genes that are important for deciding the neuronal cell types and states of *C. elegans*. To do so, we expanded the gene panel to sixty genes covering ion channels (calcium and potassium channels), neurotransmitters and neural transmitter receptors(acetylcholine, dopamine, serotonin, tyramine/octopamine, gamma-aminobutyric acid, and glutamate). With this expanded gene set, we performed ExSeq on entire intact *C. elegans* across developmental stages, obtaining high-quality RNA sequencing reads.

Future analysis and mining of the dataset will reveal how neuronal cell transcriptomic states evolve throughout development in the spatial context of the entire intact animal. This study demonstrates the feasibility of using ExSeq for spatial transcriptomics and highlights its potential as a powerful tool for functional genomics and gene expression studies in *C. elegans*. The results of this study provide a valuable resource for the scientific community and open up exciting new avenues for the study of the *C. elegans* transcriptome.

97 **Loss of CDK-4 drives nucleolar size and anabolic metabolism via lin-35 and efl-1** Rachel Webster[1,2], Maria Quintana[3], Ran Kafri[1,4], Brent Derry[1,21]Molecular Genetics, University of Toronto, [2]Developmental and Stem Cell Biology, The Hospital for Sick Children, [3]Mechanisms of Disease, IRB Barcelona, [4]Cell Biology, The hospital for Sick Children

An outstanding question in biology concerns mechanisms of size control in organisms, cells, and organelles. We recently found that cyclin-dependent kinase 4 (CDK-4) functions to regulate cell size in vitro, independent of cell cycle progression. Canonically, CDK4 in complex with Cyclin D is required in the transition from G1 into S phase of the cell cycle, but more recently it has been implicated in insulin homeostasis and fatty acid oxidation in some mammalian tissues. What remains unclear is whether these metabolic functions of CDK4 are a consequence of cell cycle progression, or if this is a distinct process related to other functions, such as size control.

We take advantage of the genetic tools available in *C. elegans* to address the role of CDK-4 in metabolism and cell size control.

I developed a system to investigate its function in cell cycle and metabolic activity using hypodermal seam cells. Seam cells contribute to cuticle production during larval development and form alae upon terminal differentiation. Most importantly, these cells divide well into larval development, consistently culminating in a syncytium with 16 nuclei. This system relies on the number of resulting nuclei as a readout of cell division, and the size of each nucleolus approximating metabolic activity. Given that the nucleolus is the site of ribosomal subunit assembly, its size is an established correlate of the rate of active protein translation in a cell. Using this system, I have shown that CDK-4 regulates nucleolar size in a way that can be uncoupled from its role in cell cycle progression. Despite being two independent processes, the downstream regulation of metabolism driven by CDK-4 appears to be mediated via the canonical LIN-35(pRb)/EFL-1(E2F) axis. Using direct measurements of protein translation, ATP levels and oxygen consumption I have confirmed that these changes in metabolism are observable on the scale of a whole worm. Consequently, lifespan and healthspan are shortened in worms with high metabolic rates, demonstrating an overall detrimental effect on the organism.

In humans, activating mutations in CDK4 are common drivers of tumor development, with CDK4 inhibitors being proven to be successful in the treatment of these cancers. Increasing usage of these inhibitors necessitates a deeper understanding of the physiological functions of this pathway, which is well conserved from worm to human.

98 **Impact of High Dietary Glucose on Aβ-induced proteotoxicity in *C. elegans*** Emylee A Kerslake, Andy Lam, Jessica E TanisBiological Sciences, University of Delaware

Alzheimer's disease (AD) is the leading neurodegenerative disorder worldwide, with an estimated 50 million individuals currently afflicted. Pathological features of this debilitating condition include amyloid-beta (Aβ) accumulation, bioenergetic defects, increased oxidative stress, and impaired glucose metabolism. Since there is currently no disease-modifying treatment for AD, it is essential to understand how modifiable risk factors such as diet impact disease onset and progression. It is difficult to determine the impact of specific nutrients in humans due to complex diet, organismal complexity, genetic diversity, and indirect effects of the gut microbiome. Individuals with abnormal blood sugar levels and glucose utilization are at greater risk for AD, likely because glucose is required to fuel neuronal function. Yet we lack an understanding of how the interplay between glucose and other macro/micronutrient availability impacts brain health. To define how diet impacts Aβ proteotoxicity we use *C. elegans* that express toxic human $A\beta_{1\text{-}42}$ in the body wall muscles, which induces robust time-dependent paralysis, reduced ATP levels, and increased reactive oxygen species (ROS). We discovered that glucose supplementation accelerated paralysis in Aβ animals that consumed OP50 *E. coli* yet had no effect on worms fed HB101 *E. coli*. While vitamin B_{12} can protect against Aβ-induced proteotoxicity, B_{12} is not the factor in the HB101 diet that nullifies the toxic effects of excess glucose levels. To determine how this diet was protective we performed RNA-Seq and observed downregulation of the predicted facilitated glucose transporter *F14E5.1 (fgt-2)* in animals fed HB101. Loss of *F14E5.1* slowed Aβ-induced paralysis, bioenergetic defects, and ROS accumulation in Aβ animals fed OP50. In the presence of excess glucose, the *F14E5.1(tm3206)* mutation abrogated accelerated Aβ-induced paralysis, resulting in a similar time to paralysis regardless of the diet consumed. These findings suggest that *F14E5.1* impacts Aβ-induced proteotoxicity, potentially by modulating glucose metabolism.

99 **A primordial TFEB/TGFβ axis systemically regulates stem cell quiescence, activation, and regeneration in the adult reproductive diapause** Tim J Nonninger[1], Jennifer Mak[1], Birgit Gerisch[1], Christian Latza[1], Klara Schilling[1], Kazuto Kawamura[1], Adam Antebi[1,21]Molecular genetics of ageing, Max Planck Institute for Biology of Ageing, [2]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Disease (CECAD)

Upon prolonged fasting, organisms remodel metabolism and can enter long-lived quiescent states such as diapause to outlast adversity. *C. elegans* adult reproductive diapause (ARD) is a long-lived adult quiescent stage that survives months without food, yet upon refeeding, animals regenerate, reproduce, and live normal lifespans. HLH-30/TFEB is a master regulator whose mutants live mere days in ARD and don't recover upon refeeding, but downstream mechanisms are unknown. Here we find that mutations downregulating TGFβ signaling, can restore *hlh-30* recovery, germline stem cell proliferation and reproductive competence. *hlh-30* induces a senescent-like DNA damage, immune and growth metabolic signature reversed by reduced TGFβ signaling. Upon fasting, HLH-30/TFEB(+) normally downregulates the TGFβ expression in sensory neurons and its receptor in the germline stem cell niche, to inhibit Notch signaling and promote ARD reproductive quiescence. Upon refeeding, these pathways are upregulated, to reactivate germline stem cells and promote reproduction. Thus, TFEB/TGFβ axis relays systemic signals linking nutrient supply to growth signaling, regulating stem cell dynamics and longevity.

100 **Preferential autophagy of ribosomes balances a trade-off between starvation survival and starvation recovery** Joel Tuomaala[1], Julie Perey[1], Siva Sankar Devanarayanan[2], Jörn Dengjel[2], Benjamin Towbin[11]University of Bern, [2]University of Fribourg

To sustain vital processes under starvation, animals degrade their biomass by autophagy. Whereas the starvation-induced auto-

phagy is required for starvation survival, extended autophagy can also be fatal if it leads to the degradation of essential proteins. Here, we asked if *C. elegans* mitigates this threat by preferentially degrading non-essential proteins over essential ones.

By combining quantitative mass spectrometry and live imaging, we found that during L1 starvation autophagy of ribosomes (ribophagy) was two times faster than autophagy of the average cytoplasm. This preference is consistent with a reduced demand for ribosomes in the absence of growth. The selective decline in ribosomes during starvation was fully dependent on autophagy genes and was quantitatively reduced by a gain-of-function (gf) mutation of *raga-1*/RagA that hyperactivates mTOR, inhibits autophagy, and upregulates biosynthesis. Consequently, *raga-1*(gf) mutants were sensitive to starvation of more than three days, suggesting that slowing down ribophagy indeed impairs resistance to extended starvation. However, slower ribophagy had a benefit during recovery from starvation of up to three days. When food resumed, wild type animals showed a lag in the re-start of growth that scaled proportionally to the depletion of ribosomes. This lag in growth was shorter in *raga-1*(gf) mutants, presumably because mutants retained higher levels of ribosomes.

We conclude that retaining ribosomes allows faster recovery from short starvation but prohibits survival of long starvation periods. The degree of selectivity of autophagy towards specific protein classes thereby balances a trade-off between the starvation survival rate and the speed of recovery. The best evolutionary compromise between these two tasks may depend on the average duration of starvation periods.

101 **Defining a novel homeostat that senses the temporal barriers of parental reproductive-span and sets progeny stress resistance capacity** Bennett Van Camp[1], Sean Curran[1,21]Biology of Aging, University of Southern California, [2]Molecular Biology, University of Southern California

The ability to respond to environmental stressors quickly and adequately is a core component to survival, and dysregulation of these resistance pathways can be debilitating. The cytoprotective transcription factor SKN-1 plays a critical role in response to multiple types of stress that can impact *Caenorhabditis elegans* fitness (e.g., developmental timing, reproductive output). However, activation of SKN-1 is antagonistically pleiotropic, driving multiple age-dependent pathologies. For example, as compared to wildtype animals, SKN-1 activation in early adulthood provides enhanced resistance to acute oxidative stress, but greater sensitivity in midlife. Additionally, constitutively active SKN-1 leads to early loss of motility, imbalanced lipid homeostasis and diminished lifespan. Because the negative phenotypes associated with constitutive SKN-1 activation manifest at the end of reproduction, we use long-term parental age selection of late progeny (last quartile of total progeny; > day 3 of reproduction) as a tool to investigate the differential impact of constitutive SKN-1 activation between early and late progeny across the reproductive span. Selection for >50 generations resulted in the significant suppression of multiple age-related measures of health that are dependent upon constitutive SKN-1 activation; most prominently, acute oxidative stress resistance and reproductive output. However, this age selection accompanies a cost to overall fitness, resulting in slowed developmental timing. This supports the notion that while parental age selection can potently influence health, it is not without a cost to overall fitness. Perhaps most surprising, acutely releasing the age selection resulted in instantaneous consequences on some, but not all, health parameters, of the immediate brood. This observation suggests the existence of a novel homeostatic set point that can bookmark the temporal boundaries of the parental reproductive span. These findings reshape the way we think about SKN-1 regulation and its role in homeostatic control from parent to offspring and provide additional details of its complex role in the regulation of healthspan across the lifespan.

102 **Parallel pathways for serotonin biosynthesis and metabolism in *C. elegans*** Jingfang Yu[1], Merly C. Vogt[2], Bennett W. Fox[1], Chester J. J. Wrobel[1], Diana Fajardo Palomino[1], Brian J. Curtis[1], Bingsen Zhang[1], Henry H. Le[1], Arnaud Tauffenberger[1], Oliver Hobert[2], Frank C. Schroeder[11]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, [2]Department of Biological Sciences, Columbia University

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) plays a central role in animal biology, e.g., regulating food intake(1), sleep(2), anxiety(3), learning(4), memory(5), and many of these functions are regulated via evolutionarily conserved biosynthesis and degradation pathways(6,7). We showed that in *C. elegans*, in addition the canonical biosynthesis of serotonin via tryptophan hydroxylase (TPH-1) in neurons(8,9), serotonin is also abundantly produced in non-neuronal tissues through a parallel biosynthesis pathway via phenylalanine hydroxylase (PAH-1). Using a mutant-based comparative metabolomics approach in combination of stable-isotope labeling, organic synthesis, MS and NMR spectroscopy, we further demonstrated that most serotonin in *C. elegans* is incorporated into *N*-acetylserotonin (NAS) and NAS-derived glucosides, which are retained in the worm body and further modified via the carboxylesterase CEST-4. Expression patterns of CEST-4 suggest that serotonin-derivatives are transported between different tissues. Moreover, we showed that PAH-1-dependent production of serotonin and/or serotonin-derived metabolites significantly contribute to serotonin-related behavior in *C. elegans*, including two well-described functions of serotonin signaling, egg laying(10) and exploratory behavior(11). Last, we showed that glucosides derived from bacterial indole appear to compete with production of serotonin-derived MOGLs via CEST-4, suggesting that bacterial tryptophan

degradation may interact with serotonin metabolism in *C. elegans*. Taken together, our study reveals a previously unrecognized complexity of neurotransmitter metabolism in *C. elegans*, providing a basis for further investigation and exploration of neurotransmitter signaling in this model system.

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103 **A Pantothenate (Vitamin B5) Metabolite Promotes Intestinal Peptide Secretion as part of Gut-Neural Axis-Mediated Stress Response** Andrew W Calof[1], Qi Jia[21]Department of Physiology, University of Southern California, [2]University of Southern California

Pantothenate is an essential vitamin used for macromolecular synthesis that must be acquired via diet or by the gut microbiota. We investigated the effects of pantothenate on the secretion of FLP-2, which is a neuropeptide-like protein whose release from the intestine promotes the activation of the antioxidant response. We found that either acute or chronic exposure of animals to exogenous pantothenate led to significant increases in the release of FLP-2::Venus fusion proteins from the intestine that was dependent upon the intestinal dense core vesicle fusion protein aex-4/SNAP25b. Pantothenate treatment did not alter the secretion of NLP-40 from the intestine or FLP-1 from neurons. Pantothenate-induced FLP-2 secretion was abolished in mutants lacking *F52H2*.4, which encodes a putative ortholog of the SLAC5A6 pantothenate transporter. Pantothenate is metabolized into Coenzyme A (CoA) by a conserved multi-step enzymatic pathway. The effects of pantothenate on FLP-2 secretion are not mediated by CoA since RNAi-mediated knockdown of any of the downstream enzymes (Y71H2AM.6/PPCS, F52H9.4/PPPCDC, or Y56B4A.8/PPAT) in the CoA biosynthetic pathway failed to block the effects of pantothenate on FLP-2 secretion, and exposure to CoA did not induce FLP-2 secretion. In contrast, knockdown of *pnk-1/PANK*, which converts pantothenate to 4'-phosphopantothenate abolished pantothenate-induced FLP-2 secretion. Finally, we found that acute pantothenate treatment increased intestinal ROS levels, and pantothenate-induced FLP-2 secretion was dependent upon the cytoplasmic superoxide dismutase/*sod-1*, but not on the mitochondrial superoxide dismutase *sod-3*. Together, our data indicate that pantothenate likely produced by bacteria activates the antioxidant response following its conversion to 4'-phosphopantothenate in intestinal cells, by increasing ROS levels, which promotes the selective secretion of the antioxidant peptide FLP-2 from the intestine. These results reveal a previously uncharacterized mechanism by which the gut-neural axis-mediated stress response can be controlled by bacterial metabolites.

104 **Cell type-specific activity of *hif-1* drives an organismal response to hypoxia** Ji Na Kong, Bob HorvitzHHMI/MIT

In the presence of oxygen, the evolutionarily conserved prolyl-hydroxylase EGL-9 promotes the hydroxylation and subsequent degradation of the hypoxia-inducible factor (HIF-1) transcription factor. In hypoxic conditions, HIF-1 is stabilized and drives critical cellular and systemic responses to hypoxia. How the consequences of HIF-1 activation vary among different cell types is poorly understood in any animal. We have investigated the cell-type-specific effects of HIF-1 activation in *C. elegans* neurons, muscles, and intestinal cells using single-cell RNA sequencing (scRNA-Seq) and Single-Cell Differential Expression (SCDE) analyses. By comparing wild-type, *egl-9*, and *egl-9 hif-1* mutant animals, we demonstrated cell- and tissue-type-specific transcriptional regulation in response to HIF-1. We initially focus on analyzing HIF-1-dependent responses specifically in muscle cells to better

understand how these cell types maintain their function and integrity despite naturally experiencing hypoxia in developmental and physiological contexts. By leveraging our scRNA-Seq data and approaches of classical molecular genetics, we identified *tspo-1* as a novel and evolutionarily conserved effector of the HIF-1 pathway. We showed that *tspo-1* can function in a subset of muscle cells to mediate a hypoxia response-dependent change in organismal locomotor activity. In mammals, TSPO is involved in the activity of cardiac muscle cells, where hypoxia commonly occurs in physiological contexts. However, the mechanisms involving TSPO therein, including its possible modulation by hypoxia, are currently unknown. We propose that HIF-1 activation in muscle cells might generally promote adaptive responses to hypoxia by modulating TSPO levels and thereby altering muscle physiology. Taken together, our findings provide insights into the effects of chronic HIF-1 activation on muscle function *in vivo* and demonstrate the utility of scRNA-Seq in identifying cell-specific gene regulation by the EGL-9/HIF-1 pathway.

105 **Novel tissue-specific regulation of the mitochondrial unfolded protein response** James P Held[1], Nadir H Dbouk[1], Adrianna M Strozak[1], Samantha H Schaffner[2], Lantana K Grub[2], Maulik R Patel[1,3,41]Biological Sciences, Vanderbilt University, [2]Vanderbilt University, [3]Cell and Developmental Biology, Vanderbilt University, [4]Diabetes Research and Training Center, Vanderbilt University Medical Center

Mitochondria are integral to cellular function as they are a major site of energy production, diverse metabolic processes, and involved in various signaling pathways. However, the activation of these pathways varies between organismal tissues depending on the requirements of the tissue-type. Further adding complexity, tissues are differentially exposed to insults that may negatively affect mitochondrial health. For instance, as opposed to other tissues, the intestine is directly exposed to ingested toxins and pathogens. Thus, a unique mechanism may be needed in the intestine to combat these perturbations. We previously reported on our discovery of a novel mechanism that regulates the mitochondrial quality control pathway known as the mitochondrial unfolded protein response (UPR^{mt}) in *Caenorhabditis elegans* (Held et al 2022). Herein we show that the phosphodiesterase HOE-1 regulates UPR^{mt} via its RNA processing role in the nucleus. Interestingly, HOE-1-dependent UPR^{mt} is specifically activated in the intestine. We used this unique feature of HOE-1-dependent UPR^{mt} to screen for other mutations that elicit intestinal specific UPR^{mt}. From a forward genetic screen for mutations that activate intestinal-specific UPR^{mt} we recovered three independent alleles in a metabolic pathway implicated in converting propionate to acetyl-CoA, including two gain-of-function mutations in the acyl CoA dehydrogenase encoding gene, *acdh-1*. Further characterization shows that hyperactivation of ACDH-1 is required in the mitochondria to activate UPR^{mt}. Moreover, ACDH-1 induced UPR^{mt} is dependent upon nuclear HOE-1 as depletion of nuclear HOE-1 completely attenuates ACDH-1-induced UPR^{mt}. Activation of HOE-1 dependent UPR^{mt} in the intestine results in preferential upregulation of innate immune response genes suggesting that activation of this response may be physiologically relevant to pathogen defense. In summary, we have identified a novel mechanism that regulates HOE-1-dependent UPR^{mt} in the intestine. Our findings provide novel insight into mitochondrial quality control regulation and promise to broaden our understanding of the intricacies of mitochondrial biology.

106 **The *Caenorhabditis elegans* microbiome (CeMbio) influences *Nematocida parisii* infection through nutrient limitation and inhibiting parasite invasion** Hala Tamim El Jarkass[1], Stefanie Castelblanco[2], Manpreet Kaur[3], Nicholas O Burton[4], Gerard D Wright[3], Aaron W Reinke[21]University of Toronto, [2]Department of Molecular Genetics, University of Toronto, [3]Michael G. DeGroote Institute for Infectious Disease Research & David Braley Centre for Antibiotic Discovery, McMaster University, [4]Department of Epigenetics, Van Andel Institute

The microbiome is known to play a crucial role in maintaining the health of its host, including providing protection against pathogenic infections. *Nematocida parisii* is a microsporidian pathogen that commonly infects *C. elegans* in the wild. To gain insight into how members of the native worm microbiome may protect *C. elegans against N. parisii* infection, we took advantage of the CeMbio collection of bacteria. Nematodes exposed to JUb44 (*Chryseobacterium scopthalmum)* and BIGb0170 (*Sphingobacterium multivorum)* are initially infected with high levels of *N. parisii*, yet subsequently exhibit reduced pathogen loads as early as 24 hours post infection. However, supplementing JUb44 and BIGb0170 with *E. coli* OP50 prior to pathogen exposure restored *N. parisii* growth. To uncover which nutrients are critical for *N. parisii* growth we have performed untargeted metabolomics. To determine if CeMbio strains produce molecules that inhibit *N. parisii* infection, we incubated *N. parisii* spores in bacterially conditioned media and then infected *C. elegans*. Both *Pseudomonas lurida* (MYb11) or *Pseudomonas berkeleyensis* (MSPm1) conditioned media resulted in decreased infection by destroying *N. parisii* spores. Liquid chromatography-mass spectrometry of MYb11 supernatant revealed that Massetolide E and F were responsible for reducing *N. parisii* infectivity. Excitingly, fractionation of MSPm1 supernatant has revealed that two independent molecules have anti-microsporidia activity. We then measured 53 additional *Pseudomonas spp.* strains, of which approximately half significantly reduce *N. parisii* infection. Our findings suggest that interactions between members of the native *C. elegans* microbiome and *N. parisii* are common and that these bacteria provide beneficial effects for their host.

107 **The host kynurenine pathway modulates and is modulated by gut microbes in *C. elegans*** Jack R Martin[1], Alejandra Zarate-Potes[1], Povilas Norvaisas[2], Ruhi Rezwana[1], Rajal Patel[1], Alexander Hardgrave[1], Catherine SS Au[1], Nadin Fathallah[1], Jayde

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The kynurenine pathway (KP) is the main catabolic route for the essential amino-acid tryptophan, whose metabolites are well-known in mammals for their immunomodulatory and neurotoxic effects. In bacteria, these tryptophan metabolites may be involved in quorum sensing or act as antibiotics. In C. elegans, inhibition of the kynurenine pathway was found to increase lifespan, possibly from improving availability of the essential amino-acid tryptophan, but the role of its downstream metabolites including anthranilic acid derivatives stored in lysosome-related organelles (LROs) remains elusive. Here hypothesised that LROs and the KP play a key role in C. elegans gut microbial control. We first tested this idea in the context of C. elegans gut infection by Enterococcus faecalis, before extending our study to gut commensals. We found that KP and LRO biogenesis mutants display varying levels of resistance to E. faecalis infection, and that enzymes promoting resistance or sensitivity to E. faecalis were accordingly up or down-regulated upon infection in wildtype N2. Increased resistance was associated with reduced gut colonisation by fluorescently-tagged pathogens, and reduced ability of mutant worm extracts to inhibit E. faecalis growth. Bacterial growth assays on KP metabolites and HPLC analysis of metabolites produced by wildtype and mutant worms on infection further identified KP compounds likely responsible for gut bacterial growth inhibition in vivo. We next investigated the capacity of the host KP to respond to and regulate gut commensals. Analysis of RNAseq datasets revealed that KP enzymes are differentially expressed when worms are exposed to new microbes. Using Tn7-mediated transformation, we fluorescently-tagged CeMbio community microbes and tested their ability to grow on KP metabolites or colonise the gut of worm KP mutants. We found that KP metabolites differentially impact the growth of the 46 isolates tested, and that worm KP mutants differentially allow microbes to colonise their guts. Strikingly, inhibition of one arm of the KP seemingly abrogated the ability of worms to discriminate between the gut commensals tested. Collectively, our results suggest that the kynurenine pathway in C. elegans critically modulates gut microbial populations through previously unacknowledged mechanisms.

108 **Metabolic modeling and characterization of the nematode-infecting bacterial pathogen Bordetella atropi.** Ila Peeler, Gabriella Canto-Encalada, Cristal Zuniga, Robert LuallenBiology, San Diego State University

We recently discovered a gram-negative bacteria, Bordetella atropi, capable of infecting the intestinal cells of the nematode, Oscheius tipulae. This bacteria uses a novel mechanism of cell-to-cell spreading via filamentation. Infection of O. tipulae with B. atropi reduces both host lifespan and fecundity, allowing us to characterize this microbe as a pathogen. We used a filamentation screen to identify a key gene, gtaB, in the UDP-glucose biosynthetic pathway which is necessary for the filamentous phenotype of the bacteria both in vivo and in vitro. However, we still lack in-depth knowledge about the specific of nutrient requirements of this pathogen and the metabolic triggers for filamentation in the host. Here, we developed a genome-scale metabolic model of B. atropi from the whole genome sequence. Using manual curation, validation, and application of a genome-scale metabolic model, we have identified 1,877 metabolites and 2,712 reactions for B. atropi. Additionally, we have characterized the biomass composition of B. atropi using a HPLC-amino acid analyzer and screened growth phenotypes on over 180 carbon sources and 95 nitrogen sources. Specifically, we saw dramatic differences in amino acid biomass between coccobacillia and filamentous morphologies. For example, the relative cysteine biomass increased 26-fold in the filamentous bacteria versus non-filamentous bacteria. This data was used to validate the model and support the assumption that B. atropi exists in two different physiological states: filamentous and non-filamentous. Systems biology approaches have been useful to understand cell-cell interactions at metabolic level and we applied this framework to understand B. atropi metabolism during infection. From our metabolic model of B. atropi, we will identify other key metabolites utilized by the bacterium to detect and initiate filamentation, and we plan to validate this model through targeted gene knockouts, phenotypic characterizations, and metabolite supplementation.

109 ***Caenorhabditis elegans* susceptibility to viral infection is modulated by its naturally associated bacteria** Rubén González, Marie-Anne FélixInstitut de Biologie de l'École Normale Supérieure

The microbes associated with an organism can modulate its susceptibility to viral infections. However, we have a limited understanding of which specific microbes modulate host susceptibility and of the molecular mechanisms that underlie this modulation. To study the three-way interaction between host, virus, and bacterial environment, we use *C. elegans*, its natural virus (Orsay virus; OrV), and bacteria found in natural association with the nematode.

We screened 67 bacterial clones for their effect on *C. elegans* viral infection using the *pals-5::GFP* transcriptional reporter (Bakowski et al., 2014). Compared to a reference bacterial strain (*E. coli* OP50), the majority of the bacteria reduced reporter expression, corresponding to a suppression of viral infection as confirmed by FISH staining of viral RNA. Few of the 67 tested bacteria increased reporter expression. We evaluated by FISH staining the infection of nematodes in two of the bacteria that enhanced transcriptional response. Compared to OP50, the proportion of infected animals was similar in *Comamonas* BIGb0172

but nematodes were more susceptible to virus in *Acinetobacter* BIGb0102.

In order to unveil the mechanisms by which bacteria modulate host susceptibility to viral infection, we selected three suppressing bacteria for further studies (*Chryseobacterium* JUb44, *Lelliottia* JUb276, and *Sphingobacterium* BIG0116). The repressive effect of these bacteria was dominant over that of *E. coli* OP50. We tested whether the repressive bacteria also repressed infection in mutant animals with impaired defense responses. Two of the bacteria (*Chryseobacterium* JUb44 and *Sphingobacterium* BIG0116) suppressed infection even in the hypersensitive *drh-1* background. Intriguingly, to fully suppress viral infection, one bacterium (*Lelliottia* JUb276) depended on the host DRH-1, which is required for both the host RNA interference and transcriptional immune responses to viral infection. However, *Lelliottia* JUb276 can induce resistance in *zip-1* and *rde-1* mutants, which are defective downstream of *drh-1*, in the transcriptional and RNA interference response, respectively. Through a genetic screen we identified *C. elegans* mutants that are insensitive to the suppressive effect of *Lelliottia* JUb276. We are currently characterizing the mutations.

Our findings provide new insights on mechanisms that modulate host susceptibility to viral infections and on the complex interactions between host, virus, and specific bacteria.

110 **Nematode-trapping fungi predation induces behavioral quiescence in *Caenorhabditis elegans*** TzuHsiang Lin[1,2], Han-Wen Chang[1,3], Yen-Ping Hsueh[1,2,31]Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan, [2]Genome and Systems Biology Degree Program, Academia Sinica and National Taiwan University, Taipei 10617, Taiwan, [3]Molecular Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan

In natural environment, the free-living nematode *Caenorhabditis elegans* faces predation stress from the nematode-trapping fungus *Arthrobotrys oligospora*, which produces three-dimensional adhesive networks to capture its prey. Here, we investigated the behavioral responses of *C. elegans* after being trapped by *A. oligospora*. We found that after 20 minutes of trapping, *C. elegans* displayed a sleep-like state of pharyngeal and movement quiescence. Using functional deficient mutants, we demonstrated that the acute pharyngeal pumping inhibition requires the ALA neuro and the movement quiescence requires the RIS neuron, both of which have been previously reported to control sleeping behavior. However, we found that physically constraining the nematodes with WormGlu also induces a similar behavior, suggesting that pharyngeal pumping inhibition under trapping might be mechanosensation induced. In addition, transcriptomic data from *C. elegans* trapped for 30 and 60 minutes revealed up-regulation of immune and defense response genes. Together, our data suggest that the nematode-trapping fungus *A. oligospora* induces up-regulation of *C. elegans* defense response genes, as well as sleep-like behavior through mechanical restriction and sleep-promoting neuron activation.

111 **A sleep-active neuron can promote survival while sleep behavior is disturbed** Inka Busack, Henrik BringmannTU Dresden

Sleep is controlled by neurons that induce behavioral quiescence and physiological restoration. It is not known, however, how sleep neurons link sleep behavior and survival. In *Caenorhabditis elegans*, the sleep-active RIS neuron induces sleep behavior and is required for survival of starvation and wounding. Sleep-active neurons such as RIS might hypothetically promote survival primarily by causing sleep behavior and associated conservation of energy. Alternatively, RIS might provide a survival benefit that does not depend on behavioral sleep. To probe these hypotheses, we tested how activity of the sleep-active RIS neuron in *Caenorhabditis elegans* controls sleep behavior and survival during larval starvation. To manipulate the activity of RIS, we expressed constitutively active potassium channel (*twk-18gf* and *egl-23gf*) or sodium channel (*unc-58gf*) mutant alleles in this neuron. Low levels of *unc-58gf* expression in RIS increased RIS calcium transients and sleep. High levels of *unc-58gf* expression in RIS elevated baseline calcium activity and inhibited calcium activation transients, thus locking RIS activity at a high but constant level. This manipulation caused a nearly complete loss of sleep behavior but increased survival. Long-term optogenetic activation also caused constantly elevated RIS activity and a small trend towards increased survival. Disturbing sleep by lethal blue-light stimulation also overactivated RIS, which again increased survival. FLP-11 neuropeptides were important for both, induction of sleep behavior and starvation survival, suggesting that FLP-11 might have divergent roles downstream of RIS. These results indicate that promotion of sleep behavior and survival are separable functions of RIS. These two functions may normally be coupled but can be uncoupled during conditions of strong RIS activation or when sleep behavior is impaired. Through this uncoupling, RIS can provide survival benefits under conditions when behavioral sleep is disturbed. Promoting survival in the face of impaired sleep might be a general function of sleep neurons.

112 **Gut-brain sphingolipid signaling regulates stress-induced aversive memory in *C. elegans*** Yu-Chun Wu[1], Isabel Beets[2], Chun-Liang Pan[11]Institute of Molecular Medicine and Center for Precision Medicine, College of Medicine, National Taiwan University, [2]Department of Biology, KU Leuven

Aversive associative memory is a conserved behavior and can be triggered by altered physiological homeostasis. *C. elegans* can associate physiological stress, such as mitochondrial disruption, with concurrent bacterial cues and develop aversive memory for those bacteria. However, it remains elusive what signals from damaged peripheral tissues modulate such aversive memory under stress. We find that aversive memory of *C. elegans* triggered by mitochondrial stress requires sphingosine-1-phosphate (S1P), which is produced by the sphingosine kinase SPHK-1. Genetic and pharmacological experiments reveal that SPHK-1 functions in the intestine and hypodermis, and the S1P that it produces is a key metabolic signal for aversive memory formation. Through gene expression profiling by RNA-sequencing and biochemical assays, we identified LPR-3 lipocalin as a chaperone for S1P, likely facilitating S1P access to the nervous system. We further identify the GPCR encoded by the *C24B5.1* locus as an S1P receptor, based on its similarity to human S1P receptor, its activation by S1P in cell-based assays, and its requirement for aversive memory in a key modulatory neuron, RIC. Our calcium imaging experiments indicate that S1P promotes sensory-evoked activity of RIC neurons under mitochondrial stress through C24B5.1. Our work thus elucidates a mechanism of gut-brain metabolic signaling and provides insights into the neural basis of stress-induced aversive memory. (supported by the National Science and Technology Council, MOST 110-2320-B-002-057-MY3 and MOST 109-2320-B-002 -019 -MY3)

113 **The IRHOM ortholgue ROM-4 functions with ADM-4/ADAM17 and FRM-10/FRMD8 to promote sleep during sickness** Alex M Moran-Rohacek, Jeremy J Grubbs, Bonnie M Mendelson, Michael J Iannacone, David M RaizenUniversity of Pennsylvania

Sickness behavior, which includes reduced movement, reduced eating, and sleep, is triggered by the release of cytokines, which communicate to central neurons. Biochemical studies have identified a protein complex responsible for cytokine secretion, but an *in vivo* role for this complex is lacking. Using an unbiased genetic discovery approach in *C. elegans*, we found that the gene *rom-4* is required for sickness sleep. ROM-4 is a homologue of mammalian iROHM proteins, which function in cytokine shedding. By testing worm orthologs of other components of the cytokine shedding complex, we identified an *in vivo* role for ADM-4/ADAM17 and for FRM-10/FRDM8. ROM-4, ADM-4, and FRM-10 all act in multiple tissues and upstream of EGF-receptor activation. Genetic interactions between the genes support the notion that they function together. Aside from a defect in sickness behavior, mutants in these three genes appear healthy and without developmental defects, suggesting that they mediate the release of a different cytokine from the EGF that mediates developmental signaling. We thus establish an *in vivo* role for the protein complex mediating cytokine release during sickness.

114 ***In vivo* monitoring of rapid adaptations in glycolysis in response to activity in *C. elegans* neurons** Aaron D. Wolfe[1], Philip J.S. Stork[2], Richard Goodman[2], Daniel A. Colón-Ramos[11]Neuroscience, Yale University, [2]Vollum Institute, Oregon Health & Science University

Neuronal activity entails large expenditures of energy. This energy demand is balanced by ATP produced by both glycolysis and mitochondrial pathways, but how these two processes collectively contribute to neuronal energy production has long been debated. One model, the astrocyte-neuron lactate shuttle, proposes that the bulk of glycolysis occurs primarily in support cells such as glia and results in lactate secretion, which is then taken up by neurons for use within the TCA cycle. Recent evidence that glycolysis increases within neurons upon activity has challenged this model. As such, the relationship between neuronal activity and neuronal glycolysis remains unclear due to a lack of sensitive tools for monitoring glycolysis at the single cell level and physiological models for manipulating activity of single neurons *in vivo*. Here we take advantage of the well-characterized nervous system of *C. elegans* to express genetically-encoded biosensors in the asymmetric gustatory neuron pair, ASE, which detect and activate upon changes in environmental sodium chloride via intracellular calcium signaling. To monitor glycolysis, we used the biosensor HYlight, which measures a key rate-limiting step of glycolysis in the form of the intermediate metabolite fructose-1,6-bisphosphate. By incorporating a microfluidics device to control a worm's ionic environment while imaging, we observed that the two ASE neurons have characteristic levels of glycolysis under basal conditions; upon challenge with changes in sodium chloride, the resulting increases in intracellular calcium were coupled to a robust increase in neuronal glycolysis, supporting the model that activity and neuronal glycolysis are linked. We hypothesize that this increase in glycolysis is a direct result of elevated demand for ATP that occurs due to activity, and will take advantage of the genetic tractability of this system to determine the mechanisms behind these metabolic dynamics. This work provides us with a powerful system for the integration of calcium activity and neuronal glycolysis at single cell resolution *in vivo* and will allow for characterization of the role adaptive glucose metabolism plays in maintaining neuronal function.

115 **Activity-dependent mitochondrial ros production regulates glutamate receptor delivery and exocytosis at synapses** Rachel L Doser, Kaz Knight, Ennis Deihl, Frederic J HoerndliBiomedical Sciences, Colorado State University

Excitatory synaptic transmission, plasticity and maintenance are central cellular mechanisms of neuronal learning and memory. The AMPA subtype of ionotropic glutamate receptors (AMPARs) plays an essential role in these processes. Decades of research have detailed signaling cascades and cellular mechanisms regulating synaptic plasticity but not synaptic maintenance after plasticity. Calcium signaling plays a central role in plasticity mechanisms, but how calcium and downstream signaling leads

to a stable number of AMPARs at the synapse is still a bit mysterious. Using *in vivo* imaging in intact neurons of *C. elegans*, we show that neuronal activity drives the production of reactive oxygen species (ROS) by mitochondria leading to decreased activity-dependent synaptic delivery and insertion of AMPARs. We go on to show that mitochondrial ROS production depends on calcium uptake by mitochondria via the mitochondrial calcium uniporter MCU-1. A genetic null mutant for *mcu-1* and acute pharmacological inhibition of MCU-1 both lead to excess synaptic AMPAR delivery and exocytosis perhaps due to decreased ROS signaling. To test this possible explanation, we combined acute inhibition of MCU-1 with optical activation of ROS production at mitochondria using the mitochondrial targeted photosensitizer, KillerRed. We found that optical activation of mitochondrial ROS production can compensates for excess synaptic AMPAR insertion when MCU-1 is inhibited. Interestingly, many previous studies have shown that the activity of calcium signaling effectors, such as calmodulin, protein kinase C or calcium/calmodulin-dependent protein kinase II, are affected by ROS signaling via oxidation of key residues. Our results suggest a model in which postsynaptic mitochondria initiate ROS signaling in an activity dependent manner which negatively regulates the synaptic delivery and exocytosis of AMPARs. This may be a novel mechanism for synaptic stabilization of AMPAR numbers in physiological conditions. They also suggest that excess ROS formation, either due to pathology or excessive calcium signaling as is known to happen in aging, can lead to progressive loss of AMPARs at excitatory synapses.

116 **Modular Glucosides : A novel family of microbe-derived metabolites which regulate egg-laying and touch sensitivity in *C. elegans*** Madhumanti Dasgupta[1], Julia Balch[1], Chester J.J. Wrobel[2], Jingfang Yu[2], Frank C. Schroeder[2], Michael O'Donnell-[31]MCDB, Yale University, [2]Cornell University, [3]Yale University

Microbes produce a wide range of potentially neuroactive chemicals in the gut. How these neurochemicals modulate brain function and behavior in animals is still largely unknown. Recent investigations into the metabolome of *C. elegans* has revealed a novel family of metabolites called modular glucosides (MOGLs) — small molecules derived from combinatorial assembly of building blocks produced by a combination of host and microbial metabolism. Interestingly, the first step in MOGL synthesis typically involves glucosylation of a neuroactive molecule or a neurotransmitter. For example, neurochemicals such as serotonin, tyramine, octopamine, indole and anthranilic acid are incorporated into diverse MOGLs. We have found that mutations in *glo-1*, which encodes a Rab GTPase essential for the biosynthesis of MOGLs, results in defects in reversal behaviors following anterior touch and in egg-laying behaviors. Carboxylesterases (CEST) - an expanded family of αβ hydrolases in *C. elegans*, are required for the formation of diverse ester linkages to specific MOGL subsets. We have found that *cest-1.*2, which is necessary for the synthesis of indole-, tyramine-, and anthranilic acid glucosides, exhibits similar phenotypes to *glo-1* mutants. Bacteria are the primary natural source of indole for *C. elegans*, and feeding worms on indole-deficient *E. coli* mimics the behavioral effects of MOGL-deficient worms, suggesting that indole glucosides are involved in driving these behavioral phenotypes. Interestingly, while intestinal expression of *cest-1.2* rescues egg-laying phenotypes, we have found that expression of *cest-1.*2 in both sensory neuron and the intestine is necessary to rescue anterior touch responses, indicating that indole-MOGLs may be trafficked and signal via distinct mechanisms in different tissues. Through transcriptomic analysis we have identified a candidate UDP-glucuronosyltransferase, *ugt-64,* which is highly upregulated in *cest-1.2* mutants. We hypothesize that glucosylation of neuroactive compounds such as indole may facilitate transport and enable tissue specific signaling of microbial produced compounds. Identifying the enzymes, and tissues involved in the assembly and dis-assembly of MOGLs and whether the building blocks are host- or microbe-derived can help understand the importance of this understudied family of modular metabolites in animal behavior.

117 **Elucidating Mechanisms of *Actinomyces* Mediated Neuroprotection in *C. elegans* Models of Parkinson's Disease** Sophie Ngana, Lesley T MacNeil, Michael G SuretteBiochemistry and Biomedical Sciences, McMaster University

Parkinson's disease (PD) is an adult-onset neurodegenerative disorder that is characterized by selective degeneration of dopaminergic neurons primarily in the substantia nigra pars compacta, as well as accumulation of alpha-synuclein enriched protein aggregates within neurons. The pathogenesis of PD is still not completely understood, and no treatments exist that target or alter disease progression. Obvious genetic causes are detected in only a small number of PD patients (5-10%), suggesting that environmental factors play a major role in its development. Specifically, correlative studies show that the microbiota may be one of these important environmental modifiers of neurodegeneration. However, existing microbiotal studies failed to establish causal links between bacterial molecules and the neurodegenerative process. In order to interrogate this causative relationship, it is important to identify individual bacteria influencing neurodegeneration and the mechanisms underlying this response.

The identification of genes that cause monogenic forms of PD allowed for the generation of several *Caenorhabditis elegans* models of PD including transgenic worms expressing human leucine rich repeat kinase 2 (*LRRK2*). *LRRK2* mutations are the most common genetic risk factor in both familial and sporadic PD accounting for 4% of familial and 1% of sporadic PD cases worldwide. Here, we used *C. elegans* expressing *LRRK2* protein in dopaminergic neurons to systematically test the influence of 57 bacterial strains isolated from the human gut microbiome. Of these, three *Actinomyces* species reduced neurodegeneration. Global gene expression analysis revealed *C. elegans* aspartic cathepsins were upregulated in response to neuroprotective *Acti-*

nomyces. RNAi-mediated knockdown of these aspartic cathepsins increased neurodegeneration in the *LRRK2* transgenic model suggesting their implication in neuronal health. Previous studies, in *C. elegans* and broadly, have successfully linked impairments in autophagy and lysosomal function to the pathogenesis of PD. We look to characterize the *Actinomyces*-mediated neuroprotection through its impact on the autophagy lysosomal pathway.

118 **Of Memory and Microbes: How stress regulators affect learning pathogen avoidance behavior** Rebekka Paisner[1], Andrew Gordus[21]Johns Hopkins University, [2]Biology, Johns Hopkins University

Organisms are constantly assessing their environments to ensure survival of self and progeny. Upon exposure to a toxin or a pathogen, an animal will modulate its behavior to prevent future interactions. The experience is then consolidated in the form of memory, and if by misfortune an animal is re-exposed to the negative stimulus, the animal will recall that memory and subsequently elicit motor responses that improve their fitness (behavior). This memory can be sustained into the next generation by altering the development of the progeny's nervous system, to reflect their parent's behavior. Just as experience can influence epigenetic processes in somatic cells, it can also affect the germline, thus passing on epigenetic information transgenerationally. Human studies have shown that adverse parental conditions can trigger epigenetic modifications that predispose offspring to obesity, metabolic disease, and pathological disorders. Yet, the cellular events that occur during transgenerational epigenetic inheritance (TEI) remain poorly understood. Recently, research with C. elegans has shown that pathogenic *P. aeruginosa* exposure can elicit avoidance behavior in subsequent generations. Parents exposed to *P. aeruginosa* and their progeny, have upregulated expression of the transforming growth factor *daf-7* in the ASI neuron compared to parents exposed to harmless *E. coli* and their progeny. We have found a non-epigenetic mechanism of single-generation inheritance that does not rely on small RNA signaling. *glp-4* and *glp-1* worms live significantly longer on a full lawn of *P. aeruginosa* compared to N2. These mutants have upregulated expression of the conserved detoxification regulator SKN-1 due to the presence of unprocessed yolk that would have otherwise been used for egg production. *glp-4;skn-1* double mutants rescued the learned avoidance phenotype. *skn-1* has 3 isoforms, of which *skn-1b*, is only expressed in the ASI neuron; this is the same neuron where *daf-7* is upregulated after *P. aeruginosa* exposure. I plan to assess the relationship between *skn-1b* and *daf-7*, and how this influences *P. aeruginosa* avoidance in the F1 generation.

119 **Investigating the role of dopamine signaling in skin penetration by *Strongyloides* species** Ruhi Patel, Aracely Garcia Romero, Elissa A HallemMicrobiology, Immunology and Molecular Genetics, UCLA

Skin-penetrating nematodes, including the human parasite *Strongyloides stercoralis*, are a major source of neglected tropical disease. These parasites invade hosts by penetrating through host skin. Although skin penetration is critical to parasitism, the process remains poorly understood. Here, we describe the behaviors executed by infective third-stage larvae (iL3s) of *S. stercoralis* and the rat parasite *Strongyloides ratti* while penetrating mammalian skin. Additionally, we demonstrate that skin penetration is regulated by dopamine signaling.

We first conducted a quantitative analysis of iL3 behavior on mammalian skin using an *ex vivo* assay. We found that most *S. ratti* iL3s penetrate skin from their natural host, the rat. Most *S. stercoralis* iL3s also penetrate rat skin, even though rats are not hosts for *S. stercoralis.* Using single-worm tracking, we found that iL3s push down on the skin surface with their heads almost immediately following contact. Thereafter, iL3s either initiate a penetration attempt by puncturing the skin with their heads or crawl a short distance on the skin surface. iL3s exhibit cycles of pushing, puncturing, and crawling until they enter the skin. Interestingly, most *S. ratti* iL3s that complete penetration on rat skin remain within the tissue, whereas most *S. stercoralis* iL3s re-emerge on the skin surface. These results raise the possibility that host-specific sensory cues are required to prevent re-emergence.

We then investigated the neural mechanisms that drive skin penetration. We found that exposure of iL3s to the dopamine receptor antagonist Haldol inhibited skin penetration; this phenotype was rescued by the addition of exogenous dopamine. Chemogenetic silencing of the dopaminergic neurons also inhibited skin penetration. These results uncover a role for dopaminergic neurons in initiating skin penetration. In addition, we identified a *Strongyloides* homolog of *Ce-trp-4*, which encodes a mechanosensitive ion channel; like *Ce-trp-4*, *Str-trp-4* is expressed in dopaminergic neurons. To test whether the *Strongyloides* dopaminergic neurons are mechanosensory neurons that detect skin texture, we are now disrupting *Str-trp-4* using CRISPR to determine the effect on skin penetration. Together, our results provide insight into how skin-penetrating nematodes invade hosts and could lead to the development of topical anthelmintic creams that block skin penetration and thereby prevent nematode infection.

120 **Phototransduction is mediated by cGMP pathway and GPCR kinase in the nematode *Pristionchus pacificus*** Ken-ichi Nakayama[1], Takahiro Chihara[1,2], Misako Okumura[1,21]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, [2]Program of Basic Biology, Graduate School of Integrated Sciences for Life, Hiroshima University

Light sensing is a crucial function for most organisms, which is mediated by photoreceptors such as opsins and cryptochromes.

A new photoreceptor LITE-1 and the phototransduction pathways have been identified in the *Caenorhabditis elegans*. However, LITE-1 is only conserved in the genus *Caenorhabditis*, and the mechanism of light-sensing in other nematodes remains unknown. To address this question, we used the nematode *Pristionchus pacificus*, which has been established as a satellite model organism for comparison with *C. elegans* and does not have LITE-1 and opsins. Similar to *C. elegans*, illumination with the short-wavelength (UV and blue) light induced avoidance behavior in *P. pacificus*. To reveal the mechanism of light avoidance behavior, we investigated mutants of six neurotransmitter-related genes and cilia-related genes. GABA and glutamate mutants showed defects in light avoidance, indicating that GABA and glutamate are required for light avoidance in *P. pacificus.* To identify genes involved in phototransduction in *P. pacificus*, we performed a forward genetic screening of light avoidance behavior and isolated six light-unresponsive mutants. By whole genome sequencing and analyzing the candidate genes using the CRISPR/Cas9 genome editing, we found that loss of cyclic GMP-gated channels and guanylate cyclases decreased light avoidance. Another unresponsive strain has a mutation in *Ppa-grk-2* encoding a G protein-coupled receptor kinase (GRK), which phosphorylates and desensitizes GPCRs. *Ppa-grk-2* mutants decreased light avoidance, while in *C. elegans* loss of GRKs did not show the reduction of light avoidance. These results suggest that in *P. pacificus* the conserved cGMP pathway is required for the light avoidance behavior and light detection in *P. pacificus* may be mediated by GPCR. Moreover, to identify photosensory neurons, we made reporter lines for phototransduction genes (*Ppa-tax-2*, 4 and *Ppa-grk-2*) and established calcium imaging in *P. pacificus*. We found that phototransduction genes are expressed amphid neurons and some amphid sensory neurons respond to light. These findings promote an understanding of the phototransduction pathways in nematodes.

121 **The heterochronic LIN-14 protein is a BEN domain transcription factor** Sharrell Greene[1], Ji Huang[2], Keith Hamilton[2], Liang Tong[2], Oliver Hobert[2], HaoSheng Sun[11]University of Alabama at Birmingham, [2]Columbia University

LIN-14, a nuclear DNA-binding protein, is a key regulator of temporal patterning in the nematode *C. elegans.* As a member of the heterochronic pathway, LIN-14 promotes developmental programs executed during early juvenile stage and represses later-stage programs throughout all somatic tissues. Curiously, LIN-14 is the only core member of the heterochronic pathway that lacks a sequence homolog outside the Nematoda. We used AlphaFold to predict the structure of LIN-14, and identified structural homology to the BEN domain, found in a family of DNA-binding proteins ((e.g., *Insensitive* or *Elba1/2* in *Drosophila* or the BEND3/4/5 proteins in mammals) previously found to have no nematode sequence homologs. We confirmed this structural prediction through targeted mutations of predicted DNA contacting residues, which disrupt *in vitro* DNA binding and *in vivo* function. We also identified additional proteins in *C. elegans* (e.g., SEL-7) with predicted similarities to the BEN domain. Connecting the two structurally allowed us to also conclude that through transcription and chromatin regulation LIN-14 and the BEN domain may have broad roles in temporal patterning. The structural deorphanization of LIN-14 as a BEN domain-containing protein provides new vistas on both LIN-14 protein function as well as BEN domain proteins in general. Since many BEN domain proteins are involved in controlling chromatin architecture, it is conceivable that, in addition to transcriptional regulation, LIN-14 may also play a role in chromatin organization. Since several non-nematode BEN domain-containing proteins have, like nematode LIN-14 and SEL-7, roles in temporal patterning, such function may have been the ancestral role of BEN domain proteins.

122 **Regulation of stress-induced sleep by neuropeptide NLP-67 in *Caenorhabditis elegans*** Vishnu Raj[1], Andromeda Veach[2], Han Wang[31]Integrative Biology, University of Wisconsin, [2]Unviersity of Wisconsin, [3]University of Wisconsin

Sleep is a fundamental physiological process observed across a wide range of organisms, including invertebrates and vertebrates, and is regulated by conservative genetic mechanisms. Despite extensive research, the molecular pathways that regulate sleep are still not fully understood. In many species, including mammals, *D. melanogaster*, and *C. elegans*, increased sleep has been observed following immune or environmental stress. Cellular stress-induced sleep in *C. elegans* is characterized by cessation of feeding and body movements as well as reduced responsiveness to the environment and is mostly mediated by the activation of EGF signalling in the ALA neuron. ALA expresses many neuropeptide genes, three of which have been implicated in promoting stress-induced sleep in *C. elegans*, but it is unclear if other neuropeptides in the ALA neuron also contribute to sleep regulation. In this study, we aimed to identify downstream regulators of EGF signalling that control stress-induced sleep in *C. elegans*. Using an unbiased genetic screen, we identified a neuropeptide NLP-67 as a candidate for promoting feeding quiescence during stress-induced sleep in *C. elegans*. We confirmed this by analysing nlp-67 deletion mutants and nlp-67 overexpression transgenic animals. To shed light on the NLP-67 neuropeptide signalling pathway, we further screened for alleles that strongly suppress sleep induced by *nlp-67* overexpression and identified 14 such alleles. Our ongoing investigations are focused on cloning these alleles to elucidate the molecular mechanism by which NLP-67 elicits sleep in *C. elegans*. Our findings will contribute to a better understanding of the molecular pathways that regulate sleep and the cellular responses to stress in organisms.

Keywords: *C. elegans*, Stress-induced sleep, ALA neuron, EGF signalling, Neuropeptide, NLP-67

123 **The SPN-4 RNA-binding protein promotes maternal mRNA clearance during the oocyte-to-embryo transition** Caroline Spike[1], Dylan M Parker[2], Tatsuya Tsukamoto[1], Naly Torres[2], Micah Gearhart[1], Erika Tsukamoto[1], Karissa Coleman[2], David Greenstein[1], Erin Osborne Nishimura[21]Genetics, Cell Biology, and Development, University of Minnesota, [2]Biochemistry

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The translational regulators LIN-41 and OMA-1/2 mutually antagonize one another as part of a repression-to-activation switch in oocytes. Their combined action ensures that SPN-4 expression initiates at the end of oogenesis (Tsukamoto et al., 2017). We used immunoprecipitation to purify SPN-4 from late-stage oocytes and RNA-seq to identify 728 mRNAs that associate with this cytoplasmic Rbfox-related RNA-binding protein (4-fold enrichment, $P<0.05$). Genes encoding SPN-4-associated mRNAs are enriched for expression in oocytes or embryonic germ cell lineages and a loss of function of many of these genes perturbs the early process of eggshell formation. Oocyte-expressed mRNAs that encode known SPN-4 regulators (*lin-41*, *oma-1/2*, *puf-3/11*, *puf-5*) were also identified as SPN-4-associated, suggesting the existence of regulatory feed-back mechanisms. Many SPN-4-associated mRNAs were described as rapidly cleared following fertilization (Stoeckius et al., 2014). Using single-molecule FISH, we observed that many of the SPN-4-associated mRNAs, including *lin-41*, *oma-2*, *chs-1* and *cpg-2* are cleared from early embryos in a SPN-4-dependent fashion. We propose that the *spn-4* maternal-effect embryonic lethal phenotype results in part from a major alteration in mRNA composition during the oocyte-to-embryo transition (OET). SPN-4::GFP is prematurely expressed in oocytes in *puf-3/11* double null mutants, and genetic experiments suggest that premature SPN-4 expression is a cause of the embryonic lethality (see the abstract by E. Tsukamoto et al., this meeting). Further, we find that mutations affecting the Ccr4-Not complex, which promotes mRNA deadenylation and decay, are dose-sensitive suppressors of the *puf-3/11* embryonic lethal phenotype. This result is consistent with the idea that SPN-4 utilizes the Ccr4-Not complex to target mRNAs for degradation during the OET. Ccr4-Not complex proteins are not found or found at reduced abundance in SPN-4 ribonucleoprotein complexes purified from oocytes where SPN-4-associated transcripts are stable. These results suggest a model in which SPN-4 expression initiates in late-stage oocytes, that it then associates with many maternal mRNAs, and that Ccr4-Not complex recruitment subsequently happens in the embryo to promote maternal mRNA clearance during the OET.

124 **A passive “hitch and tow” mechanism for cell shape change** Theadora Tolkin, Julia Burnett, E. Jane Albert HubbardCell Biology, NYU Grossman School of Medicine

Cell shape can be dynamic during development. Well-characterized modes of cell shape change include active mechanisms such as chemotaxis, arborization and axon extension. However, these active modes may not account for all instances of cell shape change. The *C. elegans* hermaphrodite distal tip cell (DTC) produces membrane-bound DSL-family ligands that activate a germline-expressed Notch receptor GLP-1 to promote germline stem cell fate. The DTC dramatically changes shape between the final larval stage and early adulthood, transforming from a cap-like structure into a plexus that includes long processes (Byrd et al 2014 *PLoS One*). The mechanisms that drive this cell shape change are not well characterized.

To better understand how the DTC elaborates, we established a granular timeline of DTC elaboration and tested several hypotheses regarding the timing of elaboration, the onset of adulthood and the cessation of gonad elongation. We found that DTC elaboration begins coincident with the final larval molt and just before cessation of gonad elongation. Despite the temporal relationship between the juvenile-to-adult transition and DTC process extension, we found that the key heterochronic pathway for this transition, LIN-41, does not regulate the timing of DTC elaboration. We tested mutants that alter gonad elongation and found that the status of gonad elongation affects DTC elaboration: prolonging gonad elongation delays DTC elaboration, while prematurely stopping gonad elongation induces precocious DTC elaboration.

We considered a mechanism relating DTC elaboration to gonad elongation and distal germ cell flux. Proliferating germ cells undergo a collective reversal during development relative to the gonadal distal-proximal axis: during larval stages, proliferating germ cells push the DTC forward (Agarwal et al. 2022 *Dev. Cell*), thus germ cells are moving in the same direction relative to the DTC. In adulthood, once the gonad has stopped elongating, germ cells move in a distal-to-proximal direction, away from the DTC. Using photoconversion assays, we measured germ cell flux and DTC process growth simultaneously, and we found that the DTC processes elongate at a consistent rate relative to the rate of expansion of the pool of photoconverted germ cells. We did not observe DTC processes extending beyond the photoconverted cells. Using conditional cell cycle arrest strategies, we found that cell cycle arrest interferes with the extension of DTC processes, while restarting the cell cycle permits subsequent process formation. Based on these and other observations, we propose a novel passive “hitch and tow” mechanism of cell shape change in which regions of adhesion between the DTC and individual germ cells facilitate the formation and extension of DTC processes.

125 **Spatiotemporal Dynamics of pulsatile miRNA transcription in the *C. elegans* Hypodermis** Shubham Sahu[1], Miguel Sambrano Lopez[1], Christopher M Hammell[2], Wolfgang Keil[11]PCC, UMR168, Institute Curie, [2]Developmental Genetics, Cold Spring Harbor Laboratories

The progression of *C. elegans* hypodermal stem cells (seam cells) through temporal cell fates is controlled by a network of ~20 genes that compose the heterochronic gene regulatory network (GRN). In this pathway, miRNAs function as molecular switches, downregulating their targets at specific transition points in each larval stage and spatial control of heterochronic

miRNA transcription is critical for allowing robust cell-fate transitions.

The transcription of *C. elegans* heterochronic miRNAs during larval development, including *lin-4* and *let-7*, is oscillatory, peaking once per larval stage, and phase-locked with the molting cycle. Past approaches to measuring the transcription dynamics of miRNAs have relied on fluorescent transcriptional reporters, limiting temporal and spatial resolution. We recently developed a new approach to monitor miRNA transcription in developing *C. elegans* larvae which combines using MS2/MCP-GFP-based RNA-localization with high-resolution long-term imaging with microfluidics. This approach uncovered highly synchronous pulses of transcription of the *lin-4* temporal patterning miRNA which are of surprisingly short duration in each larval stage. These pulses are generated by cooperative binding between the *C. elegans* orthologs of circadian regulators NHR-85/Rev-Erb and NHR-23/ROR to elements upstream of the *lin-4* gene (Kinney & Sahu et al. bioRxiv 2022).

Here, we show that, on finer temporal scales, *lin-4* transcription dynamics display cell-type specific variations and an intriguing "wave-like" spatiotemporal pattern within larval stages. Specifically, transcription loci of cells in the animal center "fire" first and cells at the anterior and posterior extremities fire last. This demonstrates a novel layer of variation in miRNA gene expression along the body axis. We also uncover that heterogeneity in *lin-4* transcription can be explained by the differential spatial expression of NHR-85/Rev-Erb and NHR-23/ROR in hypodermal cells throughout the anteroposterior body axis. Additionally, we quantify the expression of the downstream protein, LIN-14, to characterize the relationship between lineage descendance, cell cycle, and transcriptional timing among hypodermal cells.

Our results establish a new, highly quantitative approach for measuring live miRNA dynamics in the *C. elegans* larva and provide quantitative insights into the complex spatiotemporal regulation of miRNA transcription underlying temporal cell-fate patterning in the *C. elegans* hypodermis.

126 **Dissecting the role of the atypical E2F factor EFL-3 in seam cell development in *Caenorhabditis elegans*** Mar Ferrando Marco, Michalis BarkoulasLife Sciences, Imperial College London

How stem cells balance self-renewal and cell differentiation to achieve correct tissue formation and homeostasis is a fundamental question in developmental biology. In our lab, we use an epidermal population of stem cells in *C. elegans,* known as the seam cells, as a simplified model to investigate the biological regulation underlying stem cell behaviour. Through an RNAi screen, we identified the atypical E2F transcription factor EFL-3, an ortholog of the human E2F7, as a novel regulator of seam cell development. These E2Fs are thought to act as transcriptional repressors and their roles especially beyond classical cell cycle regulation are just starting to be understood. Using single-molecule *in situ* fluorescence hybridization and a CRISPR-engineered translational fusion, we show that EFL-3 mRNA and protein is found in seam cells with an intriguing asymmetric enrichment in anterior daughters after cell division. Lineage analysis in an *efl-3* seam cell-specific knockout suggests that seam cell hyperplasia and loss of seam cells occur at the same time in different lineages. Therefore, we propose that EFL-3 plays a dual role in the seam cells, contributing to maintenance of the seam cell fate and mediating cell differentiation. Targeted DamID and genetic analysis further suggests that the expression of putative targets and key seam cell factors, such as the GATA transcription factors *egl-18* and *elt-1,* and Wnt signalling components like the Axin homolog *pry-1,* is altered in *efl-3* mutants upon seam cell division. These results expand our understanding of the epidermal gene regulatory network and describe a previously unknown role for an atypical E2F in the context of stem cell patterning.

127 **Temperature experience is encoded in the AFD gene expression profile to drive neuronal and behavioral plasticity** Nathan Harris[1], Samuel G Bates[1], John Calarco[2], Piali Sengupta[11]Brandeis University, [2]University of Toronto

Neurons modify their transcriptomes in response to an animal's experience. How specific experiences are precisely transduced to modulate gene expression and tune neuronal functions are not fully defined. Given the complexity of the nervous system in mammals, it is challenging to quantify gene expression changes in specific neuronal subtypes in response to different aspects of an experience, and to link these expression changes to functional plasticity.

To address these challenges, we characterized the experience-dependent gene expression profile of the single AFD thermosensory neuron pair. We measured gene expression in AFD after prolonged exposure of animals to different temperatures, and at multiple timepoints after a temperature upshift, and found that gene expression in AFD exhibits bidirectional changes and distinct temporal dynamics in response to the animal's temperature experience. Via examination of endogenously tagged reporters, we found that salient features of the temperature stimulus including its duration, magnitude of change, and absolute value are encoded in the gene expression program in this single neuron type. We identified a novel transmembrane protein PYT-1 and a transcription factor DAC-1 whose specific transcriptional dynamics are essential to drive neuronal, behavioral, and developmental plasticity. In addition, we identified *cis*-regulatory elements that link stimuli to gene expression changes, and showed that mutating a single *cis*-regulatory site is sufficient to abolish the observed gene expression changes and neuronal plasticity.

Grouping of additional differentially expressed genes based on function suggests experience-dependent regulation of thermosensory signaling molecules and synaptic release machinery. We are exploring the contribution of these genes to plasticity. We also grouped experience-dependent genes into modules based on their temporal expression pattern dynamics and are using co-expression network analysis to predict and analyze the underlying transcriptional regulatory networks (see poster by Sam Bates). Finally, neither the regulatory mechanisms nor the functions of genes whose expression is higher at cold temperatures are known. We are using both forward and reverse genetic approaches to characterize these molecules. In summary, we aim to describe how unique experience-dependent gene expression programs and regulatory designs are customized in single neuron types to precisely regulate behavioral plasticity.

128 **Heterochromatin Protein 1 controls gene expression and longevity in response to mitochondrial dysfunction** Patricia De La Cruz Ruiz[1], Hayat Heluani Gahete[1], María de los Ángeles Ortega De La Torre[2], María Jesús Rodríguez Palero[1], Cristina Ayuso García[1], Shinya Ohta[3], Peter Askjaer[4], Marta Artal Sanz[11]Molecular Biology and Biochemical Engineering, Andalusian Centre for Developmental Biology. University Pablo de Olavide, [2]Molecular Biology and Biochemical Engineering, University Pablo de Olavide, [3]Biochemistry, Medical School, Kochi University, [4]AndalusianCentre for Developmental Biology. CSIC

Prohibitins (PHB) form a multimeric structure at the mitochondrial inner membrane. PHB deficiency shortens the lifespan of wild type *Caenorhabditis elegans* nematodes, but dramatically extends that of insulin signalling receptor (*daf-2*) mutants. This phenotype is accompanied by a differential induction of the mitochondrial Unfolded Protein Response (UPR^{mt}) that is attenuated in *daf-2* mutants. We identified Heterochromatin Protein Like 1 (HPL-1) as a new regulator of the UPR^{mt} and mediator of the opposing longevity phenotype caused by PHB depletion.

Under normal conditions, *hpl-1* null mutants live longer than wild type worms and show a mild induction of the UPR^{mt}, which depends on canonical UPR^{mt} transcription factors. We observed mitochondrial fragmentation and reduced respiration in *hpl-1* mutants, as well as in human cells upon Heterochromatin Protein 1 depletion, showing a conservation of function. Remarkably, *hpl-1* null mutants reduced the UPR^{mt} and increased respiration and lifespan of PHB depleted animals. Interestingly, HPL-1 was required for the increased lifespan and the attenuated UPR^{mt} of *daf-2*PHB-depleted worms.

In order to study genes targeted by HPL-1, we examined its binding profile in hypodermal tissue by DamID under non-stress and mitochondrial stress conditions in wild type and *daf-2* mutants. We uncovered ~75% of differently bound genes by HPL-1 in hypodermal cells upon mitochondrial stress and reduced insulin signalling. Our data shows for the first time a role for HPL-1 in controlling mitochondrial structure and function to modulate lifespan.

129 **Dietary restriction promotes healthspan via a glucagon-like signaling pathway in *C. elegans*** Brian Onken, Monica DriscollRutgers, The State University of New Jersey

A major goal of aging research is to understand the underlying relationship between nutritional intake, metabolism, and healthy aging. Low-glycemic index diets have been shown to reduce risk of age-related metabolic diseases such as diabetes and cardiovascular disease, and reduced caloric intake via dietary restriction (DR) increases healthspan across species. One potential approach for supporting healthy aging is via interventions that engage healthspan-promoting metabolism.

Our previous work demonstrated that DR increases healthspan in a manner that requires gluconeogenic gene expression. In mammals, the glucagon signaling pathway promotes glucose production in the liver by stimulating glycogenolysis and gluconeogenesis. We reason that glucagon signaling, like DR, may have an overall positive impact on healthspan.

To investigate this hypothesis, we screened for potential glucagon receptors in *Caenorhabditis elegans*, and found one candidate, *pdfr-1*, which is required for the induction of gluconeogenic gene expression under DR and for the long lifespan of dietary-restricted animals. Strikingly, overexpression of *pdfr-1* in the intestine, where we have previously shown DR-induced expression of gluconeogenic gene *pck-2/PEPCK*, is sufficient to extend lifespan. In the mammalian glucagon signaling pathway, the G protein alpha subunit coupled to the glucagon receptor activates adenylate cyclase, which increases cAMP levels to activate protein kinase A (PKA), which in turn inhibits glycolytic activity and promotes gluconeogenesis. We found that a *C. elegans*adenylate cyclase ortholog, *acy-1*, is required for increased lifespan under DR, similar to the requirement for candidate glucagon receptor *pdfr-1*. We also found that disruption of *kin-2*, which encodes the inhibitory subunit of the *C. elegans* PKA ortholog *kin-1*, triggers biomarkers for the DR state and results in dramatic healthspan increases that mirror those seen under DR. Strikingly, an *acy-1* gain-of-function mutant phenocopies the healthspan benefits of *kin-2* animals, and *acy-1* and *kin-2* function in the same pathway to affect healthy aging. Finally, we show that both the DR transcription factor *pha-4* and the *pdfr-1* receptor are required for enhanced healthspan in *kin-2* and *acy-1(gf)* animals, suggesting that these components make up a health-promoting pathway that is engaged under dietary restriction and that parallels the mammalian glucagon signaling pathway.

130 **Glia: cell non-autonomous regulators of metabolic homeostasis and longevity** Ashley FrakesNIDDK, National

Institutes of Health

As gatekeepers and guardians of the nervous system, glia act as first responders to disruptions in homeostasis long before patients or neurologists are aware of disease. Therefore, investigating the mechanisms by which glia sense and respond to cellular stressors (such as excess/insufficient nutrients or misfolded proteins) offers a unique opportunity to identify therapeutic targets and biomarkers for disease. We discovered that a small subset of astrocyte-like glial cells plays a central role in coordinating organismal protein homeostasis and longevity in *C. elegans*. To cope with stressors that threaten protein homeostasis cells have evolved compartment-specific stress responses such as the unfolded protein response of the endoplasmic reticulum (UPR^{ER}). The ability to mount an effective UPR^{ER} declines with age, which is likely a tipping point that drives protein aggregation, chronic ER stress, and ultimately, tissue damage and disease susceptibility. Remarkably, constitutive activation of the protective UPR^{ER} transcription factor, *xbp-1s*, in only four astrocyte-like glial cells initiated a robust cell nonautonomous activation of the UPR^{ER} in distal, intestinal cells which prevented age-onset loss of UPR^{ER}, reprogrammed systemic metabolism, and prolonged lifespan. Mutants deficient in neuropeptide processing and secretion suppressed glial cell nonautonomous induction of the UPR^{ER} and life-span extension. In recent, unpublished work our lab is working to identify specific neuropeptides that regulate this response. We are also defining the physiological stressors that activate glial *xbp-1s* and characterizing the cell non-autonomous transcriptional changes induced by glial *xbp-1s*. Together this work will establish how and when astrocyte-like glia regulate organismal ER stress resistance.

131 **A ribonuclease κ that promotes longevity by cleaving age-dependently accumulating circular RNAs** sieun S Kim[1], Seokjin Ham[1], Sung Ho Boo[2], Gee-Yoon Lee[1], Sangsoon Park[3], Yoonji Jung[1], Sujeong Kwon[1], Hae-Eun H Park[1], Eun Ji E Kim[1], Hanseul Lee[1], Wooseon Hwang[3], Eunah Kim[1], Yoon Ki Kim[1], Seung-Jae V Lee[11]Korea Advanced Institute of Science and Technology, [2]Korea university, [3]Pohang University of Science and Technology

Circular RNAs (circRNAs) are produced by back-splicing of diverse precursor mRNAs in eukaryotes. CircRNAs are relatively stable because of the lack of free 5' and 3' ends that confers resistance to exonucleases, and are generally upregulated age-dependently in multiple species. However, whether the accumulation of circRNAs plays causative roles in aging remains elusive. By performing RNA seq analysis using various ages of *Caenorhabditis elegans*, we found that the degradation rate of circRNAs declined during aging. Our gene set enrichment analysis indicated that the expression of ribonucleases generally reduced in aged animals. These data raise the possibility that age-dependent downregulation of circRNA-degrading ribonucleases may contribute to the accumulation of circRNAs. We then performed an RNAi screen targeting each of all 70 annotated ribonuclease genes, and identified *rnk-1*/ribonuclease κ whose RNAi knockdown greatly decreased representative circRNA levels. Our RNA seq analysis also indicated that knockdown of *rnk-1* RNAi increased overall age-dependently accumulated circRNAs. We showed that *rnk-1* mRNA and RNK-1 proteins were downregulated during aging, consistent with the age-dependent increase in circRNA levels. We identified two cysteine residues (C5 and C69) in RNK-1, which were crucial for cleaving circRNAs. By prerforming immunoprecipitation/mass spectrometry, we found that RNK-1 bound many proteins associated with RNA granules, including HSP-90 that is required for efficient clearance of stress granule (SG). Importantly, *hsp-90* RNAi also increased circRNA levels. These data suggest that RNK-1 and HSP-90 act together for degrading circRNAs in RNA granules including SGs. We then examined the role of RNK-1 in aging. We found that *rnk-1* RNAi shortened long lifespan conferred by various genetic mutations. Conversely, overexpression of *rnk-1* significantly increased lifespan. In addition, age-dependent declines in swimming (motility) rates were accelerated by *rnk-1* RNAi, and conversely delayed by *rnk-1* overexpression. Thus, RNK-1 is necessary and sufficient for increasing lifespan and healthspan. Next, we determined whether the function of RNASEK, the mammalian homolog of RNK-1, for downregulation of circRNAs and aging was conserved in mammals. We found that siRNA knockdown of *RNASEK* increased the levels of circRNAs in HeLa cells. We showed that CRISPR-mediated mutations in *RNASEK* accelerated cellular senescence in human IMR90 lung fibroblasts, by measuring various senescence markers. Thus, RNK-1/RNASEK is an evolutionarily conserved circRNA-cleaving ribonuclease that prevents aging in *C. elegans* and mammals. We propose that inhibition of toxic circRNA accumulation by ribonuclease κ can be exploited for devising novel strategies for healthy longevity.

132 **Stochastic, synchronized DAF-16 nuclear translocation pulses control stress-induced growth arrest in developing larvae** Burak Demirbas[1], Olga Filina[1], Timo Louisse[1], María Olmedo[2], María A. Sánchez-Romero[2], Jeroen van Zon[11]AMOLF, [2]University of Seville

In C. elegans, insulin/IGF-1 signaling (IIS) is responsible for mounting tailored responses to a broad range of external stresses, and induces developmental arrest upon high stress. The key step in IIS is translocation of DAF-16/FOXO to the nucleus, where it induces expression of stress-response genes. So far, it is assumed that the level of nuclear DAF-16 remains constant if stress conditions are unchanged. Surprisingly, when we visualized DAF-16 in individual L1 larvae exposed to constant stress, we instead observed stochastic pulses of nuclear translocation, with DAF-16 moving between the nucleus and cytoplasm in ~1hr pulses. The observed pulse dynamics was not only highly variable between individuals, but also differed qualitatively between applied stresses, ranging from stochastic oscillations (starvation) to random pulses (osmotic shock) or a single pulse of fixed duration

(heat shock). Pulse dynamics was also proportional to stress magnitude: increasing salt concentration increased the average number of pulses, while increasing temperature increased the duration of the single pulse.

Surprisingly, despite the often strong, stochastic variability between individual animals, we found that DAF-16/FOXO translocation pulses were always highly synchronized between all cells in the body. Moreover, DAF-16/FOXO pulses were directly linked to body-wide growth arrest: isolated pulses often coincided directly with transient reduction of growth, as measured by body length extension, while full growth arrest was seen only in animals with sufficiently long or frequent DAF-16 translocation pulses. The lack of such growth phenotypes in *daf-16* deletion mutants under identical stress conditions indicated that DAF-16 translocation pulses not only coincided with, but indeed caused growth arrest. In addition, we discovered that we could directly control and shape DAF-16 pulse dynamics by applying externally controlled repeating heat pulses. Using this approach, we found that expression of DAF-16 targets depended on the number and frequency of DAF-16 pulses, indicating that the observed differences in DAF-16 pulse dynamics between stresses could itself provide a mechanism for the stress-specificity of DAF-16-induced gene expression. Overall, our work identifies synchronized DAF-16/FOXO translocation pulses as a novel mechanism to mount stress-specific responses and developmental arrest with organism-wide coordination.

133 **Systematic mapping of organism-scale gene-regulatory networks in aging using population asynchrony** Matthias Eder[1], Olivier M.F. Martin[1], Natasha Oswal[1,2], Nicholas Stroustrup[1,2,31]Centre de Regulació Genòmica (CRG), [2]Universitat Pompeu Fabra (UPF), [3]Barcelona Institute of Science and Technology (BIST)

Multicellular organisms survive due to a functional inter-dependence among molecular mechanisms, cells, tissues, and organs. During aging, functional declines in one cell type or mechanism can produce broad organismal effects via thousands of interactions. Understanding such longdistance, one-to-many interactions is crucial for establishing causality in mechanistic studies of aging. Yet, it remains challenging to identify such interactions systematically.

We have developed a method we call “Asynch-Seq” that uses asynchrony in individual aging rates to map organism-scale interactions. By single-individual sequencing 3497 Caenorhabditis elegans transcriptomes across different timepoints and interventions, we have generated a gene-expression variation atlas that allows us to deconvole gene-regulatory changes in aging into three spatial scales - drift of individual transcripts, differential aging rates across tissues, and cross-tissue gene-regulatory couplings. We find that these couplings allow us to predict the gene regulatory consequences of a set of RNAi knockdowns and separate out the causally-distinct activities of lifespan-extending mutations. As consequence, we identified a cross-tissue set of co-varying genes that indicate a soma-germline gene regulatory axis to be a major driver of gene-expression variability in aging. In this way, Asynch-seq enables the systematic construction of causal models of organismal aging.

134 **Regulation of lifespan by sensory neural activity and CaMKI/IV** Ranran Zhao, Weiqi Ge, Weikang Xue, Jiaze Liu, Youngnam N. Jin, Yanxun V. YuMedical Research Institute, Wuhan University

Neuronal activity can influence a wide variety of physiological functions, including lifespan. A general decrease in neural activity has been linked to increased longevity. It is unclear which groups of neurons and how their activity regulate lifespan. Here, we show that deleting Ca2+/calmodulin-dependent protein kinase (CaMK) enhances lifespan, proteostasis and organismal thermotolerance by suppressing excitation in thermosensory neurons AFD in the nematode *Caenorhabditis elegans* when exposed to an elevated temperature. Similarly, decreasing hyperactivation specifically in AFD neurons by genetic modulations increases longevity and thermotolerance, resembling what happens in *cmk1* mutants. The beneficial effects of suppressing thermosensory neuron activity arise from the release of INS-1 neuropeptide from the thermosensory neural circuit and the subsequent activation of DAF-16 in the intestine. Our findings reveal a causal mechanism by which activity of the sensory neural circuit regulates lifespan and proteostasis.

135 **Knockdown of microtubule and lysosomal regulators alleviates embryonic lethality in a Nestor Guillermo Progeria *C. elegans* model** Adrián Fragoso-Luna[1], Raquel Romero-Bueno[1], Marion Kennel[1], Ángeles Bretón-Robles[1], Cristina Ayuso[1], Sophia Breusegem[2], Christian Riedel[3], Delphine Larrieu[2], Peter Askajer[11]Centro Andaluz Biología del Desarrollo, [2]Cambridge Institute for Medical Research, [3]Karolinska Institute

Nestor-Guillermo Progeria Syndrome (NGPS) is a premature ageing illness that affects a variety of tissues, leading to growth retardation, and severe skeletal defects. The syndrome is caused by a single amino acid substitution (A12T) in BAF1 (Barrier to Autointegration Factor 1), a highly conserved chromatin binding protein implicated in nuclear envelope (NE) breakdown, assembly and repair as well as chromatin compaction.

We have modified the *baf-1* locus in *Caenorhabditis elegans* to mimic the human NGPS mutation (*baf-1(G12T)*) to elucidate why a mutation in an essential protein expressed throughout development triggers the appearance of symptoms in children ~2 years after birth.

We report that NE levels of lamin/LMN-1 and emerin/EMR-1 are reduced in *baf-1(G12T)* mutants, whereas errors in chromosome segregation are increased. The *baf-1(G12T)* mutation reduces fertility and lifespan and accelerates age-dependent nuclear morphology deterioration. Moreover, we found that *baf-1(G12T)* mutants are hypersensitive to NE perturbations, particularly to modifications affecting lamin/LMN-1.

CRISPR-mediated gene knockout in NGPS fibroblasts unveiled a set of genes whose depletion alleviates the nuclear associated defects. When orthologs were silenced by RNAi in *C. elegans*, *lis-1*(PAFAH1B1/LIS1)*, vps-16*(VPS16)*, smu-1*(SMU1) and *rps-1*(RPS3A) reduced the embryonic lethality of sensitized *baf-1(G12T)* mutants. LIS1 is necessary for the correct differentiation and function of osteoclasts, regulating microtubule network and lysosomal dynamics. This offers a working model to explain the severe skeletal defects of NGPS patients. In support of these observations, we uncover that depletion of *dlc-1*(DYNLL2), *vps-11*(VPS11) and LINC (Linker of nucleoskeleton to cytoskeleton) complex subunits *sun-1*(SUN1) and z*yg-12*(HOOK1/2) also decreased the proportion of dead eggs of sensitized *baf-1(G12T)* worms. These results represent a first and encouraging list of candidate genes to be further explored for the development of NGPS therapies.

136 **H3K4me3 modifiers regulate proteostasis via an HSF-1-lipid metabolic axis** Bryndon J Oleson[1], Janakraj Bhattrai[1], Sarah L Zalubas[1], Emily Jiang[1], Christine C Lu[1], Tessa Kravchenko[1], Ciara Madden[1], Daphne Bazopoulou[1,2], Yuanyuan Ji[3], Jace W Jones[3], Ursula Jakob[11]University of Michigan, [2]University of Crete, [3]University of Maryland School of Pharmacy

Neurodegenerative diseases such as Alzheimer's disease and Huntington's disease are characterized by the pathological misfolding and deposition of proteotoxic proteins which ultimately result in neurodegeneration. Aging is a major risk factor for these diseases, and as a result, interventions that delay aging also slow disease progression. Our lab has recently discovered that developmental fluctuations in levels of H3K4me3, an epigenetic regulator of gene transcription, have long-lasting effects that impact longevity from an early stage of life. On this basis, we hypothesize that changes to H3K4me3 will also influence susceptibility to amyloid toxicity. In this study, we find that reduction of H3K4me3 levels (by knockdown of H3K4me3-modifying proteins) in *C. elegans* expressing amyloid-β or expanded polyglutamine (Q40) delayed the onset of paralysis induced by these proteotoxic proteins. Further, we found the protective effects of H3K4me3 modifier depletion against proteotoxicity to require the transcriptional regulator heat shock factor 1 (HSF-1), and depletion of H3K4me3 modifiers enhance HSF-1 activity. Further, we determined that under conditions of H3K4me3 depletion, HSF-1 regulates key aspects of lipid metabolism that are necessary for resistance to proteotoxicity. We found the gene *fat-7,* responsible for the production of the mono-unsaturated fatty acid oleic acid, to be upregulated by H3K4me3 depletion in an HSF-1-dependent manner and required for enhanced resistance of H3K4me3 modifier deficient animals to proteotoxicity. Lipidomic analysis under these conditions revealed a significant alteration to the composition of storage lipids such as triacylglycerides (TAGs), with H3K4me3-depleted animals containing TAGs with shorter chain, mono-unsaturated fatty acids compared to worms lacking HSF-1. We also find that in addition to lipid composition, HSF-1 regulates key processes involved in fatty acid breakdown via mitochondrial β-oxidation that contribute to the enhanced stress resistance of H3K4me3-depleted worms, as inhibition of β-oxidation with perhexiline prevented the protective effects of H3K4me3 depletion against proteotoxicity. These findings identify novel roles for HSF-1 in the regulation of lipid metabolism, and illuminate previously unknown links between histone methylation, HSF-1 activity, and lipid homeostasis that interact to combat proteotoxic stress.

137 **Olfactory chemosensation extends lifespan through TGF-β signaling and UPR activation** Evandro A De-Souza[1], Maximillian A Thompson[1], Rebecca C Taylor[21]Medical Research Council Laboratory of Molecular Biology, [2]School of Biological Sciences, University of East Anglia

Animals rely on chemosensory cues to survive in pathogen-rich environments. In *C. elegans*, pathogenic bacteria are known to trigger aversive behaviors through neuronal perception, and to activate molecular defenses throughout the animal. This suggests that neurons may be able to coordinate the activation of organism-wide defensive responses upon pathogen perception. We find that exposure to volatile pathogen-associated compounds induces cell non-autonomous activation of the endoplasmic reticulum unfolded protein response (UPR^{ER}) in peripheral tissues following *xbp-1* splicing in neurons. This odorant-induced UPR^{ER} activation is dependent upon transforming growth factor beta (TGF-β) signaling from ASI chemosensory neurons, and leads to extended lifespan and enhanced clearance of toxic proteins. Our data suggest that the cell non-autonomous UPR^{ER} rewires organismal proteostasis in response to pathogen detection, pre-empting the arrival of proteotoxic stress. Thus, chemosensation of particular odors may be a novel way to manipulate stress responses and longevity.

138 **Determining the role of calcium on the mitochondrial unfolded protein response** Suzanne Angeli[1], Anna Foulger[2], Julie Andersen[2], Gordon Lithgow[21]Molecular and Biomedical Sciences, University of Maine, [2]Buck Institute for Research on Aging

The opening of the mitochondrial permeability transition pore (mPTP) increases with age and drives the pathology of myriad age-related diseases such as heart attack, stroke, Alzheimer's disease, and Parkinson's disease . The opening of mPTP occurs

when mitochondria become overwhelmed by high levels of Ca^{2+}. The mitochondria open the mPTP in the inner mitochondrial membrane (IMM) to transport Ca^{2+} back into the cytosol, which causes the outer mitochondrial membrane (OMM) to rupture and initiate cell death cascades. Despite decades of research on the mPTP, the mechanisms of the mPTP and its downstream effects are not well understood, and therapeutic interventions are still needed for mPTP-related pathologies. To better understand the mechanisms of the mPTP using an *in vivo* model, we utilize the simple model organism *C. elegans*, which has highly conserved mitochondrial processes. Previously, we demonstrated that the opening of the mPTP leads to the activation of the mitochondrial unfolded protein response (UPR^{mt}) in *C. elegans* (Angeli et al., 2021). This finding was surprising because the UPR^{mt} is widely recognized as a beneficial and protective mitochondrial stress response. In the case of the mPTP-induced UPR^{mt}, however, the activation of the UPR^{mt} contributes to mPTP pathology. The signals from the mPTP that activate the UPR^{mt} are unknown. Given the integral role of Ca^{2+} on the mPTP, we tested whether Ca^{2+}-regulating pathways could activate the mPTP-induced UPR^{mt}. Our preliminary data identified calmodulin (CaM/*cmd-1*), a cytoplasmic and nuclear Ca^{2+}-binding and -signaling protein, as well as Ca^{2+} regulators in the endoplasmic reticulum (ER), as central to the activation of the mPTP-induced UPR^{mt}. In addition to cell autonomous activation of the UPR^{mt}, the UPR^{mt} is capable of becoming activated in a cell non-autonomous manner, leading us to hypothesize that Ca^{2+} signaling may play a role in inter-tissue mitochondrial communication. In support of this hypothesis, our preliminary studies demonstrate that pharmacological or genetic inhibition of germline reproduction, a large source of Ca^{2+} flux, suppresses the activation of intestinal UPR^{mt}. Our future directions involve continuing to understand the mechanistic role of Ca^{2+} signaling on the UPR^{mt}, which is of high biomedical importance.

139 **Neurotransmitters modulate lifespan via GPCR signaling in *C.elegans*** Jianfeng LiuHuazhong University of Science and Technology

Recent work has uncovered an increasingly important role of the nervous system in organismal aging. The nervous system can modulate aging via neurotransmission by secreting various small neurotransmitters that cell-nonautonomously impact the physiological state of distal tissues. Such neuroendocrine signals exert their prolongevity or antilongevity function via their cognate receptors in target tissues. G protein-coupled receptors (GPCRs) constitute the largest family of membrane proteins and are the most important drug targets. Although GPCRs are widely involved in nearly every aspect of cellular physiology, their roles in lifespan modulation have not been well recognized. Our work shows that several neurotransmitters modulate aging via GPCR signaling in *C.elegans*. First, we examined mutants lacking each of the major neurotransmitters in *C. elegans*, and find that deficiency in GABA signaling extends lifespan. This pro-longevity effect is mediated by the metabotropic $GABA_B$ receptor GBB-1. GBB-1 regulates lifespan through G protein-PLCβ signaling, which transmits longevity signals to the transcription factor DAF-16/FOXO. Then we identified a distinct neuroendocrine signaling circuit by which the worm nervous system senses cool environmental temperatures through cool-sensitive neurons and then signals the gut to extend lifespan. The prolongevity "cool" circuit uses the small neurotransmitters glutamate and serotonin. MGL-1, the GPCR targeted by glutamate, is required for transmitting longevity signals from IL1 neurons to the NSM neurons. SER-7, the GPCR targeted by serotonin, acts in the intestine to mediate the life span-extending effect of IL1 and NSM neurons. In our recent work, we show that food-associated odors shorten the lifespan of *C. elegans* under DR but not those fed *ad libitum*, revealing a specific effect of food odors on DR-mediated longevity. Food odors act on a neural circuit comprising the sensory neurons ADF and CEP, and the interneuron RIC. SER-5 and DOP-6 function in CEP and RIC neurons to transmit odor signals by responding to the neurotransmitter serotonin and dopamine released from upstream neurons, respectively. This olfactory circuit signals the gut to suppress DR-mediated longevity via octopamine, the invertebrate homolog of norepinephrine, by regulating the energy sensor AMPK through a Gq-PLCβ-CaMKK-dependent mechanism. Our results uncover new roles of several GPCRs targeted by specific neurotransmitters in lifespan regulation in *C. elegans*, raising the possibility that a similar process may occur in other organisms.

140 **The quality control system that monitors the secreted proteome: investigating the extracellular proteostasis network** Ivan Gallotta[1], Aneet Sandhu[2], Maximilian Peters[3], Martin Haslbeck[4], Raimund Jung[2], Sinem Agilkaya[2], Jane Blersch[2], Chaolie Huang[2], Della David[11]Babraham Institute, [2]German Center for Neurodegenerative Diseases (DZNE), [3]Department of Medical Neurobiology, Hebrew University of Jerusalem, [4]Department of Chemistry, Technical University of Munich

Maintaining the integrity of the proteome is essential for cell viability and the accumulation of protein aggregates is an inherent part of normal ageing in numerous organisms. Protein aggregation with age affects the proteome of different tissues, cellular compartments as well as in the extracellular space. Proteins are subject to constant surveillance by protein homeostasis (proteostasis) networks that monitor quality throughout the protein life cycle. Compared to the relatively constant intracellular environment, the conditions for secreted proteins in the extracellular space are harsher and low ATP concentrations preclude the activity of most intracellular protein-quality-control components. So, what happens if a secreted protein misfolds in the extracellular space? Does the cell, tissue, or organism care about misfolded secreted proteins? The pathologies of many serious human diseases are associated with the aggregation and deposition of misfolded proteins in the extracellular space, emphasising the dangers of misfolded extracellular proteins if left unchecked. Until now, due to the lack of genetically-amenable model organisms with physiologically relevant extracellular compartments, the extracellular proteostasis network remains

poorly defined and only a few bona fide extracellular chaperones and proteases have been shown to limit extracellular protein aggregation. Here, we have uncovered the extracellular proteostasis network that regulates protein aggregation outside of the cell in *C. elegans*. We discovered 57 regulators of extracellular protein aggregation, including several proteins related to innate immunity. Moreover, we found the first extracellular chaperone in *C. elegans*, able to bind and to stabilize aggregation-prone proteins. Promoting extracellular proteostasis can prolong lifespan and, notably, extracellular proteostasis components are up-regulated during the innate immune response to enhance survival. Mimicking a pathogenic attack, we found that *C. elegans* responded by increasing the expression of factors that limit aggregation of extracellular proteins. Similar to the ER unfolded protein response, the extracellular proteostasis network has a role in ageing and in the response to pathogens. Together this work reveals mechanisms used by the organism to protect its secreted proteome against aggregation and highlights intriguing connections with the response to pathogens.

141 **What sets the limits of transcription kinetics in the developing embryo?** Priya Sivaramakrishnan, John Isaac MurrayDepartment of Genetics, Perelman School of Medicine, University of Pennsylvania

Transcriptional precision is critical during embryogenesis to ensure that a single-celled zygote develops into an organism with the right numbers and types of cells. This precision is opposed by the inherent stochastic nature of transcription or noise that generates variability in RNA levels between identical cells. The *Caenorhabditis elegans* embryo is a paradigm model of developmental precision – development proceeds through an invariant lineage allowing us to connect quantitative transcript changes with cell fate decisions. We have found that early cell fate regulators in the *C. elegans* embryo are transcribed at extremely high rates. Rates are maximum at the 8-cell stage, soon after zygotic transcription begins, in all somatic founder lineages. Further, the transcription kinetics of some of these genes approach the theoretical maximum possible for RNA polymerase II (PolII). We hypothesize that high transcription rates are a potential mechanism to reduce transcriptional noise, allowing precise RNA levels to support precision in cell fate decisions. To explore the relationship between the transcription kinetics and transcriptional precision, we are performing live transcript imaging of early embryonic cell fate regulators using the MS2-MCP system. We are estimating initiation rates and measuring parameters associated with transcription noise (bursting) for high-rate transcription factors required for endoderm and muscle specification. The promoters of these high-rate genes are enriched for common core motifs including the initiator element (inr) and sites for the SP1 transcription factor. Thus, we are also investigating how the deletion of these promoter elements affect initiation dynamics and transcription bursting. To further examine the contributions of *cis*-regulatory elements and gene structure to controlling rates, we created synthetic promoters with different motif combinations in a fluorescent histone reporter construct. The presence of an inr element, a TATA box and SP1 binding sites along with endomesoderm-specific Med sites in the context of an otherwise 'background' sequence drives embryonic transcription in a lineage-specific manner. The same construct without the Med sites shows highly variable expression patterns. These studies are a step toward understanding the contribution of *cis*-regulatory elements in setting the maximum limits of PolII transcription rates during development and their role in effecting robust cell fate specification by buffering transcription noise.

142 **When a new gene learns old tricks: how a species-specific T-box gene came to play a role in the very early embryo** Emily A Baker, Alison WoollardUniversity of Oxford

T-box transcription factors are key players in metazoan development and exhibit minimal copy number variation throughout the animal kingdom. However, in the *Caenorhabditis* genus, T-box genes are gained and lost at an unprecedented scale. Among the suite of ten *C. elegans* specific T-box genes are three paralogue pairs all expressed during embryogenesis. These paralogue pairs display remarkable mutation accumulation patterns in wild populations, with one or other of the pair accumulating loss-of-function mutations, but never both. To understand what permits paralogues to be reciprocally lost in this way, we have characterised the roles of, and relationship between, one such pair: *tbx-35* and *tbx-36*.

The reciprocal nature of deleterious mutation accumulation among wild populations in the *tbx-35*/*tbx-36* gene pair suggests that they act redundantly, but *tbx-35* is involved in muscle specification during mid-embryogenesis, distinct from the role and expression of *tbx-36* in early embryos. Strikingly, we find that overlapping functionality between the two is only revealed when the environmental conditions are changed, suggesting that *tbx-36* has in fact retained a role in muscle specification, all while gaining a new one much earlier in development. We show that this new role for *tbx-36* is underpinned by its acquisition of E2F regulation, reflecting its transposition to a new chromosomal environment.

This combination of genetic events unfurling before our very eyes enables us to catch a rare glimpse inside the molecular engine of speciation. Traditionally, speciation is a process that can only be studied retrospectively in most systems, meaning that examples of causative loci that lead to speciation have been seldom defined. But the approach described here presents a unique opportunity to capture processes that precede new species divergence. And so, it is with a degree of confidence that we are able to say that wild populations like the ones we investigate here may one day emerge as distinct species, driven, at least in part, by the divergence of *tbx-35* and *tbx-36.*

143 **Developmentally programmed histone H3 expression regulates cellular plasticity at the parental-to-early embryo transition** Ryan J Gleason[1], Rena Guo[1], Christopher Semancik[2], Cindy Ow[3], Gita Lakshminarayanan[4], Xin Chen[11]Biology, Johns Hopkins University, [2]Tufts University, [3]UCSF, [4]Dana-Farber Cancer Institute

During metazoan development, the dramatic potency change from germline to embryos raises an important question regarding how the new life cycle is reset. Here, I will present a tightly regulated epigenome landscape change from the parental germline to embryos in *C. elegans*. To characterize the dynamics of endogenous histones throughout the *C. elegans* germline and early embryo, I have generated knock-in strains inserting protein translational tags at the endogenous histone H3 loci (15 copies of H3 are encoded in the C. elegans genome), as well as generating knock-out and point-mutations to study the developmental impact of the loss of tissue-specific histone incorporation. Using quantitative, live-cell imaging we have uncovered a surprising difference in the epigenome established in the germline, and maintained during early embryogenesis, which incorporates low levels of canonical H3 in favor of the histone variant H3.3. This H3.3 dominant epigenome persists in early-stage embryos until gastrulation, when the epigenome become H3 abundant again.

We further demonstrate that unlike somatic cells where all 15 copies of H3 are expressed, germ cells uniquely restrict canonical H3 expression to 4 loci. Upon cellular differentiation, we have identified a 100-fold increase in canonical H3 incorporation in the somatic cell lineage. This onset of canonical H3 incorporation in late-stage embryos correlates with a window of developmental plasticity which has been characterized in *C. elegans* embryonic blastomeres (Yuzyuk et al., 2009). To address the contribution of this increased H3 incorporation during gastrulation, I generated an endogenously encoded, mutated histone H3-H113D, to genetically alter chromatin assembly by destabilizing the H3-H4 histone tetramer at the onset of gastrulation. Utilizing a cell fate challenge assay to measure the degree of embryonic plasticity, we find embryonic plasticity is prolonged in H3-H113D mutants. Taken together, the data presented demonstrate H3 incorporation is developmentally programmed to restrict plasticity during embryogenesis and reinforce cell fate acquisition.

144 **Sexually dimorphic cell cycle regulation of quiescent neural progenitor asymmetric divisions** Carla Lloret Fernández, Michele Sammut, Joseph Gehler, Sophie PG Gilbert, Milou HM van der Lans, David J Elliott, Richard J PooleCell and Developmental Biology, University College London

In the vertebrate nervous system, neural stem cells are quiescent astrocyte-like glia that act as neural progenitors during juvenile and adult neurogenesis. How these quiescent cells control their ability to withdraw from and re-enter the cell cycle and how they retain neurogenic potential despite displaying molecular and ultrastructural features of differentiated astrocytes are open questions. The Poole and Barrios labs showed that the stably differentiated Amphid socket glial cells (AMso) act as neural progenitors during the juvenile-to-adult transition of *C. elegans* exclusively in males (Sammut *et al.* 2015). Here we address how quiescence exit from the cell cycle is regulated in a sexually dimorphic manner and what is the role of the cell cycle in establishing asymmetric cell fates.

Specifically, we show that the AMso glial cells are arrested in G0/G1 quiescence, like their vertebrate counterparts, and that the proneural gene *hlh-14*/*Ascl1* is required for neurogenesis but is dispensable for quiescence exit. In order to find molecular regulators of quiescence exit in the AMso, we combined forward genetic screening, RNA interference against cell cycle components and candidate gene approaches. We find that progression through G1/S in males depends on the canonical positive regulators *cdk-4/cyd-1* and *cdk-2/cye-1* CDK complexes, and primarily on the negative regulator *fzr-1/Cdh1,* co-activator of the anaphase-promoting complex/cyclosome (APC/C)*,* unlike other lineages of the worm where the transcriptional repressor *lin-35/Rb* plays an essential non-redundant role. Differential response to abrogation of *cdk-4* and *fzr-1* between both sexes drives G1/S entry exclusively in males. This suggests that cell cycle regulators may exist in two different states in male vs hermaphrodites. We uncover that the sex determination pathway and the cell cycle genetically interact to regulate the AMso division. Finally, we show that progression through the G2 step but not mitosis *per se* is required for the glia-to-neuron cell fate switch. We are currently investigating if neuronal specification could be mediated by the G2-to-M regulator *cdk-1*.

Our data support a model where a sexually dimorphic state of positive and negative regulators of the cell cycle differentially regulates quiescence exit in the AMso. These observations are particularly relevant in light of the reported sex differences in the incidence and outcome of glioblastoma (Yang *et al.* 2019) and the sexually dimorphic proliferation response to the loss of cell cycle genes in glioblastoma mouse models (Sun *et al*. 2014). A deeper understanding of sexually dimorphic cell cycle control in neural precursors will likely improve our understanding of the sex differences in the prevalence of brain tumours and neurodegenerative diseases.

145 **The transcription factor LSL-1 is a main player in germline/soma distinction** David Rodriguez Crespo, Magali Nanchen, Shweta Rajopadhye, Chantal WickyUniversity of Fribourg

The proper separation between soma and germline is critical for the development of sexual-reproducing organisms. Germline/soma distinction involves cell fate decision and maintenance and requires the regulation of a large gene repertoire, which is mainly achieved by transcription factors and chromatin proteins. In this context, chromatin factors such as the remodeler LET-418/Mi2 and the heterochromatin protein HPL-2/HP1 have been identified as crucial repressors of the germline transcriptional program in the soma. Lack of LET-418 or HPL-2 activity leads to ectopic P-granule assembly around somatic nuclei. On the other hand, in the germline, we characterized here a zinc-finger transcription regulator, named LSL-1 (stands for *lsy-2*-like), that activates germline genes and that is essential to maintain germ cell fate.

LSL-1 is first detected within P4-germline blastomere, when zygotic transcription is initiated, and remains present at all stages of germline development, from primordial germ cell proliferation to the end of meiotic prophase. *lsl-1* mutants exhibit defects in the progression through meiotic prophase, but more importantly a failure in the maintenance of germ cell identity. In *lsl-1* mutant gonad, germ cells are progressively reprogrammed into neurons as the worms age. Transcriptomic data and ChIP-seq results indicate that LSL-1 acts as a direct transcriptional activator of a large set of germline genes. Additionally, genetic interaction studies show that LSL-1 functions by antagonizing HPL-2 and LET-418, chromatin factors involved in the repression of germline gene expression in somatic cells. This is consistent with our observations that ectopically induced *lsl-1* is sufficient to trigger P-granule assembly in somatic cells.

Overall, we propose LSL-1 to be a master regulator of germline genes and we hypothesize that LSL-1 could be part of a system that initiates germline gene expression during development based on the epigenetic memory left in the parental germline. Currently, we are characterizing the chromatin context in which LSL-1 functions to establish germline-soma distinction in *C. elegans*.

146 **Inter-individual variation in gene expression underlies reproductive traits in isogenic worms** Amy Webster, John H. Willis, Erik Johnson, Patrick C PhillipsUniversity of Oregon

Understanding how phenotypic variation is generated for complex traits is a fundamental problem in biology. Genetic variation underlies many phenotypic differences, but phenotypic differences exist in the absence of genetic change. In particular, isogenic *Caenorhabditis elegans* nematodes exhibit substantial differences in 1) egg-laying onset timing and 2) early brood size. These traits are influenced to some extent by past and present environmental conditions, but persist even among isogenic worms in the same environment. To understand the extent to which gene expression differences may underlie these traits and act either independent of or dependent on past environmental conditions, we performed single-worm mRNA-seq on 192 individuals phenotyped for both reproductive traits at the same stage of early adulthood. These isogenic worms were in the same environment when isolated, but experienced two controlled environmental perturbations early in life: 1) they were the either the progeny of day 1 or day 3 adult mothers and 2) they experienced a constant early-life temperature or a temporary shift to 25°C as young larvae. Analysis of resulting mRNA-seq data identified hundreds of genes for which gene expression variation is associated with reproductive traits. Further analysis of this rich dataset revealed the extent to which gene expression differences are mediated by environmental effects to exert changes on phenotypes – for example, some genes associated with brood size almost completely depend on maternal age, others act independently, and some are intermediate. We also identified multiple small sets of genes that together are highly predictive of both traits, setting the stage for using gene expression as a phenotyping proxy. Finally, we chose candidate genes among those strongly associated with early brood size to validate experimentally, demonstrating that the natural expression variation identified is causal. Together, this work reveals that we can predict differences among genetically identical individuals in fitness-related traits based solely on variation in gene expression, raising the possibility that such variation is not noise per se but can be understood at a functional level.

147 **Spatial single cell sequencing of meiosis I arrested oocytes reveals acquisition of maternal transcripts from the soma** Kenneth Trimmer[1], Peisen Zhao[2], Jacob Seemann[3], Shin-Yu Chen[1], Sudip Mondal[2], Adela Ben-Yakar[2,4], Swathi Arur[31]MD Anderson Cancer Center, [2]Department of Electrical and Computer Engineering, University of Texas at Austin, [3]Genetics, MD Anderson Cancer Center, [4]Department of Mechanical Engineering, University of Texas at Austin

Maternal RNAs are stored from minutes to decades in oocytes throughout meiosis I arrest. For long, studies have suggested that during this arrest oocytes are transcriptionally quiescent. Recent reports however suggest that nascent transcription in oocytes leads to generation of maternal transcripts. Whether arrested oocytes launch nascent transcription in response to environmental or hormonal signals while maintaining the meiosis I arrest remains undetermined. We test this by integrating spatial extraction and single cell RNA deep sequencing, RNA Velocity and RNA fluorescent *in-situ* hybridization analysis on *C. elegans* oocytes at different ages during meiosis I arrest. We discovered a population of transcripts that increases as the arrested meiosis I oocyte ages but ruled out extracellular signaling, through ERK MAPK, and nascent transcription as a mechanism for this increase. Instead, we report transcript acquisition from neighboring somatic cells as a mechanism for the increase in transcripts during meiosis I arrest. These analyses provide a new view of the RNA landscape at a single-cell resolution of a meiosis I arrested oocyte and as it prepares for oocyte maturation and embryonic progression.

148 **Dynamic chromatin regulation and the *C. elegans* molting clock.** Stephen P Methot[1], Maike Graf-Landua[1,2], Dimos Gaidatzis[1], Helge Grosshans[1,21]Friedrich Miescher Institute for Biomedical Research, [2]Faculty of Natural Sciences, University of Basel

During development, cells separate regions of the genome into open euchromatin and closed heterochromatin to drive cellular identity and function. While establishment of these chromatin states is believed to occur in a progressive and linear manner, response to stress or specific temporally regulated transcription programs may involve more dynamic behaviors. To gain insight into the scope and mechanisms of such chromatin dynamics, we study the *C. elegans* molting clock, a rapid (~7-hour period) and rhythmic transcriptional program that controls the expression of ~3,700 distinct transcripts. Strikingly, using ATAC-seq, we observe wide-spread rhythmic chromatin accessibility changes, correlated with rhythmic gene expression. To understand mechanistically how these rhythmic changes to chromatin are achieved, we performed a targeted RNAi screen using developmental timing as a readout. Among the hits from the screen were the histone acetyltransferase *cbp-1* (CBP/p300) and the SWI/SNF chromatin remodeler, both associated with open and transcriptionally active chromatin. We have begun to evaluate changes to gene expression and chromatin accessibility by performing time-resolved RNA-seq / ATAC-seq following acute, degron-based depletion of these factors and will discuss our preliminary results. Additionally, we observed that their extensive depletion results in a developmental arrest, with features suggesting the existence of a chromatin integrity checkpoint. Thus, animals arrest immediately after completing a molt, and arrested animals can re-initiate development if protein levels are restored. Collectively, our data support the existence of highly dynamic rather than static chromatin and a checkpoint where its state is surveyed.

149 **Transcriptional Control of a developmental transition in *Caenorhabditis elegans*** François-Xavier Stubbe[1], Florian Steiner[2], Damien Hermand[11]URPHYM-GEMO, UNamur, [2]Dept. of Molecular and Cellular Biology, University of Geneva

Transcription of protein coding genes is carried out by the RNA polymerase II (Pol II). It is now clearly established that Pol II itself is subject to many modifications that can influence how factors required for transcription and RNA processing are recruited. The largest subunit of Pol II harbors an unstructured tail like C-terminal domain (CTD) composed of repeats of the consensus heptapeptide sequence $Y_1S_2P_3T_4S_5P_6S_7$. A large body of work showed that the phosphorylation status of the CTD changes in a predictable pattern as Pol II moves along the transcription unit. Pol II is recruited to transcription units with a hypo-phosphorylated Pol II and becomes heavily phosphorylated first on serine 5 (CTD-S5) during the transition from initiation to early elongation, and then on serine 2 (CTD-S2) during productive elongation. While the CTD can be modified in many additional ways on all of its residues, the anti-correlated gradient of CTD-S5 and CTD-S2 phosphorylations (CTD-S5P, CTD-S2P) are the most conserved and best characterized marks.

We previously generated an analogue sensitive (-as) version of the cyclin-dependent kinase 12 (CDK-12as). Inhibition of CDK-12as by an ATP analogue caused a significant drop of CTD-S2P indicating, along with additional evidence, that CDK-12 is the main CTD-S2 kinase. Remarkably, embryogenesis occurs normally in absence of detectable CTD-S2P but leads to a L1 arrest in the F1 of inhibited worms. Genome-wide analyses indicated that when CTD-S2P is inhibited, a subset of growth-related genes is not properly expressed. Those genes correspond to downstream (position 2 or over) genes within co-transcribed cluster of 2 to 8 genes called operons. Contrarily to most genes that are capped with a splice leader 1 (SL1) RNA, downstream genes within operons receive a 5' cap by *trans-splicing* to a SL2 RNA. We showed that CTD-S2P is a key component of the control of development and has a specific role in the expression of SL2 trans-spliced mRNAs while not globally required for gene expression. We demonstrated that CstF is recruited less efficiently when CDK-12 is inhibited. As CstF was shown to bring the SL2 RNA, it provides a molecular basis for the specific *trans-splicing* defect caused upon CDK-12 inactivation. To better characterize the mechanism at play, we set up a straightforward suppressor screen. We identified an allele of the polyadenylation factor and CTD-S5P phosphatase *ssup-72* that robustly suppresses the L1 arrest induced by CDK-12as inhibition.

150 **LincRNAs Promote Germline Stem Cells Differentiation via Sequestering PUF Proteins to Phase-Phase Condensates** Roni Falk, Hanna Achache, Yonatan B TzurDepartment of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem

Optimal fertility depends on a tight balance between germ line stem cells proliferation and differentiation. Two evolutionary conserved PUF proteins, FBF-1 and FBF-2, maintain proliferation and delay meiotic initiation in progenitor germ cells of *C. elegans*. The FBF reduce the stability of mRNA from thousands of meiosis promoting genes. However, it is unclear how meiosis initiates even though the FBF are present at significant levels not only in early germline stem cells but also in late proliferative and early meiotic stages.

We hypothesized that three long-intergenic-non-coding RNAs (lincRNAs) that bind the FBF proteins act to restrict FBF action in late proliferative and early meiotic stages. We found that the expression of the lincRNAs increase during late proliferative and

leptotene/zygotene cells. Deletion of the lincRNA genes leads to additive reduction in proliferative cells and progeny number. We found similar effects in worms with extra copy of FBF-2, suggesting that without the lincRNAs, FBF-2 is overactive. In the triple lincRNA mutant the expression of many known FBF-2 targets is significantly reduced, supporting the possibility that the lincRNAs restrict FBF-2.

We show that the lincRNAs promote the aggregation of FBF-2 to peri-nuclear phase separated condensates. The number of FBF-2 condensates increase as the cells move out of the germline stem cell niche, concurrent with the increase in lincRNA abundance in these aggregates. The relative level of free cytoplasmic FBF-2 is also reduced just before meiotic entry. Deletion of the lincRNA genes prevent this change in FBF-2 cellular localization and lead to aberrant meiotic entry. Moreover, previous reports suggested that association of FBF-2 with the P-granules, an evolutionary conserved peri-nuclear condensate, is critical for meiotic entry. Without the lincRNA this association is significantly reduced.

Taken together, our results point towards an evolutionary conserved mechanism for lincRNAs in repressing PUF proteins, by restricting them into phase-separated granules, and thus enable timely meiotic entry.

151 **The USTC complex defines the chromatin environment of piRNA genes in the *C. elegans* germ line** Nancy Sanchez, Lauren Gonzalez, Valerie ReinkeGenetics, Yale University

The Piwi interacting RNA (piRNA) pathway is a conserved small RNA pathway that maintains genomic fidelity of the germ line by silencing foreign genetic elements and inappropriate gene expression. In *C. elegans*, >10,000 sequence-diverse piRNA genes cluster in two distinct megabase-scale regions on a single chromosome. Despite their high expression, these piRNA genes are located in repressive chromatin domains. piRNA expression is promoted by the upstream sequence transcription complex (USTC), composed of PRDE-1, SNPC-4, TOFU-5, and TOFU-4, which binds strongly across the piRNA gene cluster. The functional relationship between the USTC complex and chromatin environment remains poorly understood, in part because previous *C. elegans* chromatin studies were performed in whole animals and lacked germline-specific resolution. To achieve germline-specific chromatin profiles, we optimized a method to isolate germ nuclei to perform ChIP-seq and ATAC-seq in germ nuclei. We then tested whether the USTC complex delineates specialized domains that facilitate or permit piRNA transcription by establishing germline specific chromatin states at piRNA genes in the germ nuclei of wild-type and *prde-1* mutants, a mutant that disrupts USTC complex formation. Strikingly, we observed that in wild-type germ nuclei, piRNA genes reside between nucleosomes and that repressive modifications are depleted locally at piRNA promoters. In *prde*-1 mutants, the overall nucleosome environment at piRNA genes is less structured and the chromatin is less accessible at piRNA gene transcription start sites. By performing H3 Native ChIP-seq in wild-type and *prde-1* mutant germ nuclei, we observed that PRDE-1 is required to establish a strong H3 signal specifically upstream of piRNA genes. These data also showed that overall density of H3 across the piRNA domains also depends on PRDE-1. This result suggests that the USTC complex plays a role in both promoting nucleosome density and organizing the local nucleosome environment to direct the exposure of piRNA genes to transcriptional activators within genomic regions that are otherwise enriched for repressive chromatin marks. We are currently investigating how the USTC complex interacts with chromatin remodelers and transcription initiation regulators. Overall, this work reveals a new mechanism for how transcriptional regulation is coordinated over large genomic domains, which has implications for understanding global genome organization in the germ line.

152 **Mitopherogenesis: an unexpected mitochondria-specific ectocytosis process and its critical role in modulating mitochondrial content and fertility of sperm** Hongyun Tang[1], Peng Liu[2], Jing Shi[1], Danli Sheng[11]School of Life Sciences, Westlake University, [2]HangZhou XiYue Biotechnology Co., LTD

Mitochondrial export into the extracellular space is emerging as fundamental cellular processes implicated in diverse physiological activities. Although a few studies have shed light on the process of discarding damaged mitochondria, how mitochondria can be exported and the physiologic functions of mitochondrial release remain largely unclear. In particular, whether there exists mitochondria-specific export mechanism is unknown. Here, we report mitopherogenesis, a formerly-unknown process that specifically secretes mitochondria out of the cell through a unique extracellular vesicle termed “mitopher”. We observed that during sperm development, healthy mitochondria are exported out of the male gametes in *C. elegans* through mitopherogenesis and each of the generated mitophers harbors only mitochondrion but no other organelles. Time-lapse imaging indicated that in mitopherogenesis, the mitopher is released immediately from the cell periphery upon being loaded with one mitochondrion into the plasma membrane outward bud. Furthermore, we have identified the extracellular proteases in the testis acting as the developmental cue to trigger mitopherogenesis and the Fer tyrosine-kinase-SPE-8 critically mediate this process. Moreover, we found that mitopherogenesis requires normal microfilaments dynamics and identified the translocating-motor that modulates mitophers generation. Strikingly, our 3D-EM analyses showed that mitochondrial quantity requires precise modulation during sperm development, which is critically mediated by mitopherogenesis. Inhibition of mitopher generation causes sperm fertility defect likely due to the disturbed mitochondrial content. Our findings identify mitopherogenesis as a previously-undescribed

process for specifically exporting mitochondria, which may represent a fundamental branch of mechanisms underlying mitochondrial quantity control to regulate cell functions during development.

153 **A Bipartite Nuclease and Cytidine Deaminase is Required in the Soma and the Germline to Promote Transgenerational Male Fertility** Nicholas Galambos[1], Colin Conine[21]Biology, University of Pennsylvania, [2]University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia

The ALG-3/4 26G-RNA pathway is a specialized endo-siRNA pathway that operates exclusively during male gametogenesis in *C. elegans* to promote thermotolerant male fertility. We have recently found that *nyn-3* encodes a bipartite protein that contains a ribonuclease domain (NYN domain), which is essential for ALG-3/4 26-G RNA production, and for the generation of functional sperm at elevated temperatures. Additionally, NYN-3 contains a cytidine deaminase domain of unknown function. Remarkably, we've found that the cytidine deaminase domain functions in somatic cells to promote thermotolerant male fertility transgenerationally. *nyn-3 cytidine deaminase* catalytic mutants and soma-specific knockdown of NYN-3 produce males and hermaphrodites that progressively lose fertility at 25° due to defective sperm. Interestingly, while small RNA-seq of *nyn-3* cytidine deaminase catalytic mutant males revealed no changes in ALG-3/4 26-G RNAs when compared to wildtype animals, we observed a dramatic increase in *ergo-1* dependent 22G-RNAs. *ergo-1* functions, in a once thought distinct, small RNA pathway specific to oocytes, embryos, and somatic tissues. Our results support a model where the cytidine deaminase of *nyn-3* functions in somatic cells, negatively regulating ERGO-1 22G-RNA synthesis. This somatic regulation appears to be critical for the transport of an unknown signal to the germline, which ensures transgenerational male fertility. These findings suggest a novel role for RNA-editing in regulating endogenous small RNA pathways, soma-to-germline communication, and transgenerational fertility. Given the conservation of small RNA and Argonaute pathways in the male germline of all animals, this study offers a broader perspective on how RNA processing and somatic contributions can influence male fertility and paternal epigenetic inheritance in other species.

154 **Live single-molecule imaging shows localized translation of endogenous transcripts in the *C. elegans* embryo** Elise van der Salm, Esther Koelewijn, Erica van der Maas, Bas van Dorst, Suzan RuijtenbergUtrecht University

Translation regulation determines the timing, efficiency and location of protein synthesis from an mRNA and is essential for gene expression control. Translation regulation is particularly important during early stages of embryogenesis when transcription is silenced and gene expression fully relies on post-transcriptional regulation. Although translation regulation is clearly important, many aspects contributing to development have remained elusive.

To study how translation is regulated during development, we created a live-imaging method to visualize translation dynamics in *C. elegans*, based on the previously described SunTag system. First, we integrated 24 SunTag repeats downstream of the start codon of an endogenous transcript or reporter. Next, we co-expressed a GFP-labeled SunTag antibody that binds SunTag peptides with high affinity as soon as they emerge from the ribosomal exit tunnel during translation, resulting in the accumulation of the GFP signal at sites of translation. Indeed, bright SunTag translation spots were observed throughout *C. elegans* development by live imaging. These Suntag spots overlapped with mRNAs and disappeared upon inhibition of translation, indicating that they indeed represent active sites of translation.

Using our newly developed SunTag system, we aimed to understand the molecular mechanism and importance of localized translation. For this, we focused on mRNAs that have been shown to co-localize with their encoded protein, such as the polarity proteins DLG-1, AJM-1 and ERM-1. As expected, analysis of the endogenously *SunTag-tagged erm-1* showed that many translation sites are found close to the cell cortex, co-localizing with the ERM-1 protein. To study the influence of localized translation on protein function, we are combining the SunTag system with mRNA labeling/tethering techniques to visualize and change the localization of mRNAs. Indeed, our data show that by combining these techniques we can manipulate the localization of translation, allowing us to address the importance of localized translation.

Taken together, we have successfully developed the SunTag system in *C. elegans* and shown its functionality in studying when and where endogenous mRNAs are translated in embryos and larvae. Our recently developed SunTag-based tools will provide novel insight into translation heterogeneity and spatiotemporal control of translation during development.

155 **Tug of war between PAR-3 and CDC-42 in the polarisation of the *C. elegans* zygote** Josana Rodriguez, John Packer, Elise Benett, Adam WollmanBioscience, NUBI, Newcastle University

Most cells are polarised, meaning that they present structural and molecular asymmetries essential to their function, for example the barrier function of epithelial cells in tissues, or the generation of different cell types from asymmetrically dividing cells. The polarisation of cells relies on polarity effectors, among them the PARs, that specify distinct membrane domains. Domains are typically defined by mutual antagonistic interactions between different sets of PAR proteins, where by, for example, the set of PARs that define the anterior of the cell will exclude the posterior PARs and vice-versa. More recently we have revealed that in

addition to this mutual antagonism, complex functional relationships exist between PARs acting within a given domain. Studying the asymmetrically dividing *C. elegans* zygote we proposed the existence of a dynamic exchange of PAR-6/aPKC between PAR-3 and CDC-42. Through super-resolution imaging we have captured this exchange and gained a mechanistic understanding of how this exchange controls PAR-6 dynamics and organisation at the membrane, revealing a tug of war between PAR-3 and CDC-42 in the polarisation of the *C. elegans* zygote. Moreover, we have found that this tug of war is regulated by the activity of the aPKC kinase per se. Overall, we have identified novel regulations between a conserved set of polarity regulators, known to act in conjunction in many cell types, hence we expect our findings to widely impact the understanding of cell polarisation.

156 **Multiple pathways for re-establishing PAR polarity in *C. elegans* embryo** Laurel A Koch, Lesilee S RoseMolecular and Cellular Biology, University of California, Davis

Asymmetric cell division is the process by which one cell divides to give rise to cells with different fates, and it is important for generating cell diversity during development. Intrinsically asymmetric division requires a polarized axis along which cell fate determinants are localized. In many organisms, polarity is established by the PAR proteins, which accumulate in mutually exclusive domains in response to a symmetry breaking cue. In the first division of the *C. elegans* embryo, the centrosome-associated Aurora A kinase, AIR-1, acts as that cue. AIR-1 inhibits actomyosin at the future posterior pole, resulting in anteriorly directed cortical flow that moves the initially uniform anterior PAR proteins (aPARs) to the opposite pole. Posterior PAR proteins (pPARs) then move onto the cortex. Mutual antagonism between aPARs and pPARs maintains these domains. While polarity establishment and maintenance in the one-cell embryo (P_0) have been well studied, less is known about how reciprocal PAR domains are formed in subsequent germline P cell divisions such as the P_1 cell. Prior work revealed movement of the nuclear-centrosome complex towards the posterior of the P_1 cell and anteriorly directed actomyosin and aPAR flow. To test the hypothesis that a centrosome-based cue triggers P1 polarity, we used live cell imaging of fluorescently-tagged proteins to determine the timing of polarity establishment relative to AIR-1 localization and actomyosin flow. We found that at the end of P_0 cytokinesis, PAR-2 (a pPAR) was present uniformly around the cortex. A posterior PAR-2 domain started to form within 2 minutes, before nuclear movement and actomyosin flow, and when AIR-1 was in a diffuse cloud. To identify other mechanisms for early P_1 polarization, we examined mutants for the kinases PKC-3 and PAR-1. In *pkc-3(ts)* embryos at permissive temperature, the PAR-2 domain never formed, while in *par-1* null and kinase-dead mutant embryos, the PAR-2 domain formed late. Analysis of additional *par-1* mutants and downstream effectors suggest that PAR-1's role in generating cytoplasmic polarity is key to proper P_1 cell polarization. Finally, *par-1(RNAi)* in combination with either reduction of centrosomal AIR-1 or myosin activity resulted in more severe polarity defects. We propose a model where PAR-1 and PKC-3 are required for early P_1 polarization, while AIR-1 and actomyosin flow act in a redundant pathway that can generate polarity with a temporal delay.

157 **The mechanical role of the nucleus in cell invasion in *C. elegans*** Johan d›HumièresEcole Normale Supérieure

Cell invasion is the penetration and migration of cells across a special type of extracellular matrix barrier, basement membrane, which separates different tissue compartments. It is a critical step in non-pathological contexts such as immune surveillance and also in various pathologies, including cancer cell metastasis. The biochemical aspects of cell invasion have been well-studied, but the mechanical aspects are less well-known. Here we study an invasion event, anchor cell invasion, which occurs during the development of the nematode *Caenorhabditis elegans.* Due to the simplicity of genetically modifying and imaging the worm, this approach allows for a quantitative evaluation of the mechanics of cell invasion.

Previous work from the team showed that the actin-rich invasive protrusion of the anchor cell applies forces on the extracellular matrix to break through it. Here we address the role of the anchor cell nucleus, its link to the cytoskeleton and how the nucleus participates in invasion. Our hypothesis is that as the largest and stiffest organelle in a cell, the nucleus could play a key role in the mechanical aspects of invasion.

We find that removing the protein components that link the actin and microtubule cytoskeletons to the nucleus (the LINC complex) one by one leads to reduced invasion in 20% of the worms examined, while removing multiple components or using a dominant negative approach increases the invasion defect to 40-50%. Whereas wild type anchor cell nuclei are highly deformed during invasion, nuclei with altered connection to the cytoskeleton are perfectly round as compared to wild type. We observe a similar round nucleus phenotype by reducing the actin network. Overall, our results indicate that the cytoskeleton is applying forces on the anchor cell nucleus during invasion and that those forces are important for invasion efficiency.

158 **An intrinsic polarising signal for asymmetric cell division in the nematode genus *Auanema*.** sally adams[1], Talal Al-Yazeedi[2], Sophie Tandonnet[1], Anisa Turner[1], Jun Kim[3], Junho Lee[3], Andre Pires da Silva[11]The University of Warwick, [2]Liverpool School of Tropical Medicine, [3]Research Institute of Basic Sciences

Asymmetric cell division (ACD) is a key biological process that generates daughter cells with different fates and is essential for cell diversity and embryonic development. Intrinsic or extrinsic polarising signals regulate the differential segregation of cyto-

plasmic components to produce these distinct daughter cells, though the role of intrinsic factors is not well understood. The nematode genus *Auanema* provides a unique opportunity to study the role of intrinsic factors in ACD. Sex determination in *Auanema* is regulated chromosomally; females and hermaphrodites have two X chromosomes (XX) while males inherit only one (XO). During *Auanema* male spermatogenesis, essential sperm components segregate asymmetrically with the X chromosome, while non-essential cytoplasmic materials are disposed of in the nullo-X sister cell, that takes the role of a residual body. Thus, crosses between males and females results in few sons, as the males produce mostly X-bearing sperm. Combining different genetic backgrounds disrupts ACD, resulting in an increased number of functional nullo-X spermatids and an increase in male progeny. This provides an easy-to-score phenotype to study ACD at the organismal level. We hypothesise that the X chromosome acts as an intrinsic polarising signal during *Auanema* male spermatogenesis. Our observation that crossing genetically distant strains of *Auanema freiburgensis* produces lines that segregate as either low (~10%) or high (~50%) male allowed us to identify a region on the X chromosome important for asymmetric spermatogenesis through Quantitative Trait Locus (QTL) mapping. Furthermore, we used the unique father-to-son inheritance of the X chromosome in *Auanema* to introgress the X chromosome of one strain into the autosomal background of another strain. X introgression generated high male-producing lines, suggesting the interaction between strain-specific autosomal and X chromosome components plays a role in maintaining asymmetric spermatogenesis. Identification of these autosomal factors will allow us to further elucidate the role the X chromosome plays in ACD.

159 **Connecting neighboring epithelial cells to make one continuous digestive tract** Lauren Cote[1], Abigail Converse[1], Melissa Pickett[2], Jessica Feldman[11]Biology, Stanford University, [2]San Jose State University

Epithelial cells form continuous barriers such as the gut and skin, protecting animals from external environments. In *C. elegans*, the digestive tract forms an internal tube with one continuous apical surface running from mouth to anus, and ultimately joins with the skin to create an epithelial toroid. This continuous epithelial surface encases the entire animal and is composed of cells from multiple lineages. How different, neighboring epithelial tissues connect and align to form this epithelial toroid is poorly understood. Development of the *C. elegans* digestive tract requires epithelial connections both within a single tissue and epithelial connections across different tissues. The *C. elegans* intestine polarizes through the action of the conserved scaffolding protein PAR-3 and the E-cadherin homolog HMR-1. Once polarized along the apicobasal axis, the intestine must then connect to neighboring epithelial tissues to form one continuous apical surface along the digestive tract. Our work suggests that the rectal valve cells form a bridge between the gut and the invaginating epidermal-like rectal epithelium. Cell ablations of the rectal valve precursor results in the formation of a closed off intestinal cyst instead of a connected tube. Unlike the well-characterized anchor cell-vulval epithelial connection, the rectal cells never breach a basement membrane. Instead, the two rectal valve cells concentrate conserved apical polarity proteins in two large apical puncta. These apical puncta occur at the interface of both neighboring tissues, the intestine and the rectal epithelium. Using tissue-specific protein degradation and laser cell ablations, we found that formation of apical puncta within the valve cells depends upon intestinal E-cadherin/HMR-1 but not intestinal PAR-3. These data indicate that intestinal adhesion complexes instruct cell polarization of the neighboring valve cells. In addition to exploring how tissues instruct each other during the development of the digestive tract, we are also exploring a newly identified model of repair of congenital epithelial connection defects. We found that although depletion of intestinal PAR-3 during development leads to a population of arrested, cystic L1 animals (Pickett et al., 2022), late re-expression of PAR-3 after epithelial polarization allows for the repair of the cystic gut into a continuous tube in larvae. ~30% of cystic L1 larvae survive to become fertile adults by 6 days post hatching after late re-expression of PAR-3 (2-fold stage). We are actively investigating whether intestinal adhesion complexes and/or spatial information from neighboring tissues influence this model of epithelial repair.

160 **Investigating FGF dispersal and the spatial organization of downstream signaling proteins in migrating cells** Theresa Gibney, Laila Latifi, Ariel PaniDepartment of Biology, University of Virginia

Cell signaling dynamics are often tightly regulated to affect cell migration, proliferation, and differentiation. We are using *C. elegans* postembryonic muscle progenitors as a tractable model to dissect cell-cell signaling mechanisms *in vivo*. One muscle progenitor type, the sex myoblasts (SMs), migrate from near the tail of the worm to the center during larval development. Fibroblast Growth Factor (FGF) signaling is required for SM migration, and FGF has been hypothesized to act as a chemoattractant for migrating cells. FGFs are often thought of as soluble signaling proteins that can diffuse to form gradients. However, extracellular diffusion has not been observed with endogenous FGFs, and in several contexts FGFs are membrane anchored proteins that signal at cell contacts. To investigate how FGFs guide SM migration, we tagged the endogenous FGF (EGL-17) involved in SM migration and imaged its dynamics along with membranes of FGF-expressing cells and migrating SMs. Unexpectedly, we did not observe an extracellular FGF gradient from the center of the worm, but instead observed FGF expressed by ventral cells along the route taken by migrating SMs. However, live imaging of FGF along with ligand and receptor expressing cells, in their natural context and in misexpression experiments, demonstrated that EGL-17 is diffusible *in vivo* and spreads between cells that are not in contact. We then anchored endogenous EGL-17 to source cell membranes, which disrupted SM migration and confirmed that free FGF

dispersal is required despite the absence of a visible extracellular gradient. Misexpression experiments are also consistent with diffusible FGF acting as a directional cue for SMs. To further investigate how extracellular FGF is translated into directed cell migration, we are also characterizing and manipulating the localization of downstream components that link activated FGF receptors to intracellular signal transduction pathways. We identified one regulatory protein involved in FGF signal transduction that localizes to the leading and ventral edges of migrating SMs, and its redistribution or SM-specific depletion results in disrupted SM migration. This work provides direct evidence that an endogenous FGF acts as a diffusible signaling protein and provides new insights into mechanisms that translate extracellular growth factor signaling into directed cell behaviors.

161 **Independent regulation of mitochondrial DNA quantity and quality in *C. elegans* primordial germ cells** Aaron ZA Schwartz[1], Nikita Tsyba[2], Yusuff Abdu[3], Maulik Patel[4], Jeremy Nance[31]Cell Biology, NYU School of Medicine, [2]Vanderbilt University, [3]NYU School of Medicine, [4]Vanderbilt Universtiy

Mitochondria harbor an independent genome, called mtDNA, which contains essential metabolic genes. Although mtDNA mutations occur at high frequency, they are inherited infrequently, indicating that germline mechanisms limit their accumulation. To determine how germline mtDNA is regulated, we examined the control of mtDNA quantity and quality in *C. elegans* primordial germ cells (PGCs). We show that PGCs combine strategies to generate a low point in mtDNA number by segregating mitochondria into lobe-like protrusions that are cannibalized by adjacent cells, and by concurrently eliminating mitochondria through autophagy, reducing overall mtDNA content two-fold. As PGCs exit quiescence and divide, mtDNAs replicate to maintain a set point of ~200 mtDNAs per germline stem cell. Whereas cannibalism and autophagy eliminate mtDNAs stochastically, we show that the kinase PINK1, operating independently of Parkin and autophagy, preferentially reduces the fraction of mutant mtDNAs. Thus, PGCs employ parallel mechanisms to control both the quantity and quality of the founding population of germline mtDNAs.

162 **A Conserved Requirement for RME-8/DNAJC13 on Neuronal Autolysosome Reformation** Sierra Swords, Nuo Jia, Anne Norris, Jil Modi, Qian Cai, Barth GrantRutgers University

Autophagosomes fuse with lysosomes, forming autolysosomes that degrade engulfed cargo. To maintain lysosomal capacity, autolysosome reformation (ALR) must regenerate lysosomes from autolysosomes using a membrane tubule-based process. Maintaining lysosomal capacity is required to maintain cellular health, especially in neurons where lysosomal dysfunction has been repeatedly implicated in neurodegenerative disease. The DNA-J domain Hsc70 co-chaperone RME-8/DNAJC13 has been linked to endosomal coat protein regulation and to neurological disease. We report new analysis of the requirements for the RME-8/DNAJC13 protein in neurons, focusing on intact *C. elegans* mechanosensory neurons, and primary mouse cortical neurons in culture. Loss of RME-8/DNAJC13 in both systems results in accumulation of grossly elongated autolysosomal tubules. Further *C. elegans* analysis revealed a similar autolysosome tubule accumulation defect in mutants known to be required for ALR in mammals, including *bec-1/beclin* and *vps-15/PIK3R4/p150* that regulate type-III PI3-kinase VPS-34, and *dyn-1*/dynamin that severs ALR tubules. Clathrin is also an important ALR regulator implicated in autolysosome tubule formation and release. In *C. elegans* we found that loss of RME-8 causes severe depletion of clathrin from neuronal autolysosomes, a phenotype shared with *bec-1* and *vps-15* mutants. We conclude that RME-8/DNAJC13 plays a previously unrecognized role in autolysosome reformation, likely affecting ALR tubule initiation and/or severing. Additionally, in both systems, we found that loss of RME-8/DNAJC13 appeared to reduce autophagic flux, suggesting feedback regulation from ALR to autophagy. Our results connecting RME-8/DNAJC13 to ALR and autophagy provide a potential mechanism by which RME-8/DNAJC13 could influence neuronal health and the progression of neurodegenerative disease.

163 **Non-random segregation of mitochondria during asymmetric neuroblast division** Ioannis Segos, Jens Van Eeckhoven, Barbara ConradtCell and Developmental Biology, University College London

Mitochondria cannot be generated *de novo* and thus their partitioning is regulated during cell division. Recently, it was reported that mitochondria can be partitioned asymmetrically during stem cell divisions in mammals; however, the mechanisms and functions of this asymmetric inheritance are poorly understood. In addition, asymmetrically inherited mitochondria have been suggested to influence cell fate determination, but the mechanisms involved remain unclear.

To study mitochondrial partitioning, we have developed a method that enables us to systematically follow the same cell division in *C. elegans* L1 larvae at single organelle resolution. At least to our knowledge, this has never been done before. Our method is based on the mechanical and drug-free immobilization of L1 larvae, super-resolution live imaging and 3D rendering of cells and mitochondria. Using this approach, we discovered that mitochondria are asymmetrically partitioned during the division of the neuroblast QL.p. QL.p divides to generate a smaller cell fated to die (QL.pp), and a larger cell fated to survive (QL.pa). During QL.p division, most mitochondria are inherited by the surviving QL.pa, resulting in increased density in QL.pa and decreased density in QL.pp. We also have evidence that mitochondria are asymmetrically partitioned by morphology, with an enrichment of smaller round organelles in QL.pp (see poster by Jens Van Eeckhoven). Conversely, elongated mitochondria, with a higher

membrane potential, are exclusively inherited by QL.pa. Therefore, there is also a functional asymmetry between mitochondria inherited by the two QL.p daughters. Moreover, we have discovered that mitochondrial dynamics is required for mitochondrial partitioning. Disrupting either *fzo-1* (required for mitochondrial fusion) or *drp-1* (required for mitochondrial fission) function results in abnormal mitochondrial partitioning. Based on these observations, we propose that mechanisms exist that control mitochondrial morphology and partitioning during QL.p division, thereby enabling asymmetric mitochondrial inheritance.

We are currently performing long-term imaging experiments in super-resolution to determine whether asymmetric mitochondrial partitioning contributes to cell fate determination in QL.pp and QL.pa. The ultimate goals of this project are to obtain a mechanistic understanding of asymmetric mitochondrial inheritance during animal development and to determine how it can influence daughter cell fates.

164 **Phospholipid flippases regulate glial phagocytosis of a sensory neuron ending** Violet Sorrentino[1,2], Aakanksha Singhvi[1,21]Basic Sciences, Fred Hutch Cancer Research Center, [2]Molecular and Cellular Biology, University of Washington

The nervous system contains two cell types, neurons and glia. Maintenance of neuron ending architecture, where neurons receive neuronal or environmental input, is critical for a healthy nervous system. One way glia contribute to this is through pruning, whereby glial cells phagocytose fragments of living neuron endings. How glia determine which parts to engulf, or how much, is not well-understood. Our lab previously established *C. elegans* as a model to study regulation of glial pruning.

One neuronal signal that glia recognize to initiate pruning is phosphatidylserine (PS). PS is an “eat me” signal known to facilitate phagocytic clearance of cell corpses. In apoptosis and other cellular contexts, exposure of PS and other phospholipids is controlled by P4-ATPases called flippases. However, it remains unknown what flippases are involved in glial pruning. We previously identified a role for the flippase TAT-1/ATP8A, which controls PS exposure, during AMsh glial pruning of the AFD sensory neuron ending. We now report that loss of CHAT-1/CDC50A, the obligate chaperone for TAT-1, phenocopies the glial pruning defect of *tat-1* mutants, further suggesting that maintenance of PS asymmetry is critical for regulating pruning. Accordingly, both *chat-1* and *tat-1* mutants have abnormally long engulfed AFD fragments persisting in glia. We infer that glia rely on TAT-1/CHAT-1-dependent PS decoration to determine which parts of the neuron ending to engulf.

We also report a novel role for the phosphatidylethanolamine (PE) flippase TAT-5/ATP9A, which is implicated in EV release and endosomal recycling in other contexts. Contrary to *tat-1*, *tat-5* mutants exhibit reduced pruning, as well as increased frequency of vesicle-like structures surrounding the AFD ending. Cell-specific rescue experiments show that TAT-5 acts in glia. Surprisingly, CRISPR-based structure-function analysis suggests that TAT-5 does not require the DGET motif for glial pruning. Together, these results lead us to hypothesize that glial TAT-5 facilitates uptake of neuronal fragments through a novel mechanism. We are currently examining TAT-5 biology in glia, and whether PE regulation broadly impacts pruning. Interestingly, mammalian ATP9A is implicated in neurological defects through unknown mechanisms. Our work introduces an exciting facet to understanding how PS/PE lipid asymmetry in both neurons and glia can influence glial pruning, and consequently neuron architecture and function, in both health and disease.

165 **Cilia-intrinsic mechanisms and not neuronal transduction regulate dynamic release of extracellular vesicles (EVs)** Juan Wang[1], Josh Saul[2], Inna Nikonorova[1], Carlos Nava Cruz[2], Maureen M Barr[21]Rutgers University, [2]Genetics, Rutgers University

Extracellular vesicles (EVs) are membrane-bound particles that can mediate long-distance, cross-organ communications between cells. For example, cancer cells secrete EVs to facilitate organ-tropic metastasis [1]. In *C. elegans* six IL2s in both sexes and 21 male-specific neurons release EVs from cilia into the environment for inter-organismal communications [2-4]. We previously showed PKD-2, a transient receptor potential polycystin-2 channel, is a conserved ciliary EV cargo. Using in vivo imaging of fluorescently-tagged proteins, we showed that ciliary EVs are produced at the ciliary base and tip [5]. Ciliary tip EVs are released directly into the environment. Isolated EVs trigger behavioral changes in males. During mating, males transfer PKD-2::GFP-labeled EVs to the cuticle of the hermaphrodite vulva. How cell signaling influences EV production is a fundamental question in cell biology.

Here, we investigated the time scale of PKD-2 ciliary EV release. We imaged cilia releasing PKD-2::GFP-tagged EVs in real-time by taking time-lapse movies. The ciliary tip released EVs at a rate of 1-2 EVs per minute. Males mounted under a glass slides released PKD-2 EVs continuously for an hour. Over one hour, *C. elegans* males exhibited a three-fold increase in PKD-2::GFP ciliary EV release. Disrupting neuronal transduction in *unc-13* or *unc-31* mutants did not affect EV release initially or after one hour, suggesting that neuronal transduction does not regulate ciliary EV release. The transition zone protein NPHP-4 was not required for initial EV release but was essential for sustained ciliary EV release. In contrast, the ciliary kinesin-3 KLP-6 was required for both initial and sustained ciliary EV release. In summary, we discovered that cilia are capable of dynamically releasing EVs. Our long-term goal is to use *C. elegans* as a living animal model to reveal the basic principles of signal-induced EV biogenesis and

EV-mediated intercellular communication.

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166 **Lysosomes contain an expansion compartment that mediates zinc transporter delivery to promote zinc homeostasis in *C.elegans*** Adelita Mendoza[1], Nick Dietrich[1,2], Chieh-Hsiang Tan[1,3], Daniel Herrera[1], Jennysue Kasiah[1], Zachary D Payne[1], Ciro D Cubillas[1], Daniel D Snyder[1], Kerry D Kornfeld[11]Washington University, [2]NIH, [3]California Institute of Technology

Zinc is a transition metal that is essential for all life. Conserved zinc homeostatic mechanisms exist to respond when cytosolic levels are too low or too high. The Kornfeld lab uncovered high and low zinc homeostasis pathways in *C. elegans* that are involved in sensing cytosolic zinc levels and responding to return to homeostasis. Zinc dyshomeostasis leads to multiple human pathologies.

Intestinal lysosomes are a site of zinc trafficking. The zinc exporter, CDF-2 stores zinc within lysosomes. We predicted that a mechanism exists to release zinc from lysosomes since there is one that stores it. To identify the zinc transporter that imports zinc we performed confocal fluorescence microscopy and showed that ZIPT-2.3 co-localizes with CDF-2. To determine ZIPT-2.3 function, we performed genetic and biochemical assays to show that ZIPT-2.3 imports zinc into the cytoplasm, and that the *zipt-2.3* gene is necessary for this function in zinc deficient conditions. Because CDF-2 and ZIPT-2.3 co-localize to gut granules, we tested their relationship by measuring their changes in expression in zinc replete, deficient, and excess conditions. Analysis showed that *cdf-2* and *zipt-2.3* mRNA and CDF-2 and ZIPT-2.3 protein expression is reciprocally regulated. Super resolution microscopy revealed that in addition to housing CDF-2 and ZIPT-2.3, lysosomes alter their morphology in response to available cytosolic zinc. Using line scans, we defined specific compartments and membrane boundaries across zinc conditions. Lysosomes contain acidified and expansion compartments. ZIPT-2.3 and CDF-2 populate membranes that surround the acidified compartment, while CDF-2 is localized to the expansion compartment. The acidified compartment is comprised of distinct regions named the LysoTracker and zinc regions.

The expansion compartment is highly dynamic and deviates greatly in size. Our model predicts that in zinc deficient conditions, ZIPT-2.3 expression increases and CDF-2 expression decreases, promoting the net flow of zinc into the cytosol. In zinc excess conditions, CDF-2 expression increases, and ZIPT-2.3 expression decreases, facilitating net flow into the gut granule. The expansion compartment expands in zinc excess conditions but shrinks in zinc deficient and zinc replete conditions. Based on our observations we conclude that the purpose of the expansion compartment is to provide a structure for zinc trafficking while allowing the lysosome to participate in its canonical activities. Future studies will focus on how the expansion compartment is formed and if expansion compartment form in human lysosomes.

167 **Neural control mechanisms of sex pheromone-triggered mate-searching behavior** Xuan WAN[1], Vladislav Susoy[2], Aravinthan D.T. Samuel[2], Paul Sternberg[31]BBE, caltech, [2]Harvard, [3]BBE, Caltech

The ancient role of the olfactory system in animals' communications with complex signals in the environments is fundamental to well-being, reproduction, and pathological states. Sex hormones contribute to specialized sexual behaviors in most animals in nature. There is a knowledge gap of how odor perception is encoded spanning across different levels, including the molecular mechanisms, neural substrates, and neural computations that guide precise behavioral programs. In this talk, we will present how brain integrates this information to guide olfactory navigation with the full spectrum of cells' activity that responds to sex pheromone perception, which enables high-efficiency spatial searching and sexually dimorphic behaviors.

It is universal across organisms that searching for specific targets, such as food or mates, requires an array of coordinated behaviors, including (1) active sampling, (2) strategic navigation, and (3) decision-making. (1) Sex-pheromone chemoreceptor SRD-1

exhibits an intriguing sexually dimorphic expression pattern—expression only in head neurons in hermaphrodites, but expression in an additional male-specific neuron PHD in the tail. Our calcium-imaging results showed that PHDs are activated by crude sex pheromone and that this perception is SRD-1-dependent. Thus, we hypothesize a novel mechanism for odor localization in *C. elegans*: males compare signal inputs from spatially separated detectors and integrate this information to guide olfactory navigation, enabling high-efficiency spatial searching and sexually dimorphic behaviors. (2) We also observed a novel navigation strategy in *C. elegans*: worms switch between three distinct navigation modes (orthokinesis, klinokinesis, klinotaxis) during pheromone- and other odor-prompted navigation behavior. Worms compare stimulus intensity between sequential single point measurements to regulate speed (orthokinesis) and/or turning rate (klinokinesis). Alternatively, animals also move their sensory organ to detect spatial patterns (klinotaxis). (3) Sexually dimorphic behaviors can be elicited by pheromones without any learning and also can be further shaped by life experience. We observed that pheromone-specific habituation, sex, development stage, energy state, and reproductive mode contribute to variations in odor preferences.

168 **Sensory cilia architecture and intraflagellar transport shape sensory signaling** Alison Philbrook, Laura Grunenkovaite, Piali SenguptaBiology, Brandeis University

Morphologically diverse sensory cilia are essential for sensing a range of environmental stimuli including odorants. Sensory cilia are formed by the conserved process of intraflagellar transport (IFT) that transports ciliary proteins, including structural and signaling components, into and out of cilia. Cilia house all signal transduction molecules including receptors and channels and thus play critical roles in shaping sensory responses. However, how distinct cilia morphologies contribute to sensory signaling is not well understood. Chemosensory neurons in *C. elegans* exhibit a range of morphologically unique cilia, and each neuron type responds to a defined set of chemicals. Using genetics, *in vivo* calcium imaging, and high-resolution behavioral assays, we have found that IFT and cilia morphologies differentially contribute to individual sensory neuron responses and chemosensory behaviors. As mutations in IFT genes result in severely truncated cilia, we generated a temperature-sensitive mutation in a *C. elegans* kinesin motor gene to decouple the contributions of cilia morphology and IFT to odorant responses. After acute shift of kinesin-*ts* mutants to the restrictive temperature, IFT is blocked but cilia morphology remains intact. We have found that a minimum cilium length but not IFT regulates chemosensory responses in neurons with rod-like simple cilia, whereas cilia morphology and IFT differentially regulate olfactory response dynamics in neurons with complex cilia. Morphological complexity appears critical for sensory responses to pyrazine in AWA complex cilia, whereas mutants with severe cilia structural defects can still detect diacetyl through upregulation of the diacetyl receptor ODR-10 in the AWA sensory endings. However, AWA cilia are required for the correct organization and concentration of regulatory components including the GRK-2 GPCR kinase such that desensitization of the diacetyl response is affected in cilia mutants. Additionally, regulated IFT-mediated ciliary trafficking of ODR-10 likely drives adaptation to diacetyl, whereas adaptation to pyrazine appears to require a different mechanism. Our work describes how specialized cilia morphologies contribute to sensory responses of individual chemosensory neurons in *C. elegans,* and highlights the importance of these structures in precisely shaping sensory behaviors in an odorant-specific manner.

169 **NeuroSCAN: a tool for analyzing neuronal relationships across scales and throughout development** Noelle L Koonce[1], Sarah E Emerson[1], Mark W Moyle[1], Pura Arroyo-Morales[1], Nabor Vázquez Martínez[1], William A Mohler[2], Daniel A Colón-Ramos[1,31]Cell Biology and Neuroscience, Yale University School of Medicine, [2]Genetic and Developmental Biology and Center for Cell Analysis and Modeling, University of Connecticut Health Center, [3]Wu Tsai Institute

NeuroSCAN is a web-based resource designed to enable community-wide examination of *C. elegans* neuronal relationships in the nerve ring. It facilitates comparisons across spatial and temporal scales— from systems level changes in the positions of neurons with respect to each other in the neuropil, to cell level morphological changes to the nanoscale positioning of synapses between neurons. Using electron microscopy datasets of the nerve ring throughout larval development, we have derived three-dimensional representations of the connectome, contactome, and 'neuron clusters' (White et al., 1986; Moyle et al., 2021; Brittin et al., 2021; Witvliet et al., 2021). Neuronal clusters, or groupings, were derived using network algorithms that revealed structurally and functionally relevant fasciculating neighborhoods throughout development. These neuronal relationships can now be explored, enabling users to examine morphologies of cluster member-neurons, neuron-neuron contact sites, and synaptic positions between partners, all in the context of the nerve ring. NeuroSCAN also features modeled representations of neuron contact profile 'patches' across development to enable unrestricted exploration of the neuropil's network architecture. In combination with other existing tools in the community (WormATLAS, NemaNode, CytoSHOW, WormWiring), NeuroSCAN facilitates web-based inspection of the nervous system, enabling multi-scale analysis and visualization of neuronal relationships throughout larval development.

170 **Structural insights into the formation of repulsive Netrin guidance complexes** Jessica M Priest[1], Ev L Nichols[2], Jesse B Hopkins[3], Juan L Mendoza[4], Kang Shen[2], Engin Ozkan[11]Department of Biochemistry and Molecular Biology, Institute for Neuroscience, and Institute for Biophysical Dynamics, University of Chicago, [2]Howard Hughes Medical Institute and Department of Biology, Stanford University, [3]The Biophysics Collaborative Access Team (BioCAT), Argonne National Laboratory, [4]Department of

Biochemistry and Molecular Biology and Department of Biochemistry and Molecular Biology, University of Chicago

The wiring of the nervous system is under strict control during development. Cell-surface receptors bind to extracellular guidance cues, directing neurons to their intended targets. Netrin (UNC-6 in nematodes) is a heavily studied bifunctional guidance cue, capable of attracting and repelling axons by binding to different receptors. The role of Netrin goes beyond axon guidance, contributing to cell proliferation, migration, differentiation, and survival. DCC receptors (UNC-40 in nematodes) are required for both attractive and repulsive responses, while UNC-5 receptors are required only for repulsive responses. Molecular details of Netrin–UNC-5 interactions and how they signal remain elusive as there is limited biochemical characterization and structural information. Here, we showed that nematode UNC-5 is a heparin-binding protein and determined its structure bound to a heparin fragment. We used directed evolution strategies and structure-based rational design to modulate the UNC-5–heparin affinity, confirming that heparin or related glycosaminoglycans (GAGs) are needed for high-affinity UNC-5–UNC-6 interactions, while a weaker complex can be formed in the absence of them. Furthermore, nematode UNC-5 and UNC-6/Netrin form a large, stable, and rigid oligomeric complex in the presence of heparin (~1.5 MDa), which can incorporate the attractive UNC-40/DCC receptor, demonstrating binary and ternary ectodomain complexes at preparative scale for the first time. *C. elegans* with a heparin-binding deficient UNC-5 fail to establish proper gonad morphology due to abrogated distal tip cell migration, which relies on repulsive UNC-5 signaling in response to UNC-6. However, the weaker-affinity UNC-5–UNC-6 complex appears to be sufficient for axon guidance, as ventrally localized UNC-6 can still repel UNC-5 expressing neuronal commissures towards the dorsal side in these mutant animals. This observation of two distinct phenotypes may be a result of several mechanistic explanations including: (1) UNC-5-mediated cell migrations may depend on the higher affinity of heparin-mediated UNC-6–UNC-5 interactions while a weaker affinity in the absence of heparin is sufficient for axon guidance functions, or (2) heparin-mediated oligomerization of the UNC-6–UNC-5 complex is necessary for signaling during distal tip cell migrations. Our findings establish that Netrin responses may require large signaling complexes mediated by GAGs/proteoglycans, depending on the context.

171 **UNC-43/CaMKII regulates synapse assembly in *C. elegans*** Mizuki Kurashina, Kota MizumotoZoology, University of British Columbia

Neurons communicate via a specialized interface known as the synapse comprised of a presynaptic and postsynaptic specialization. While the structural and functional components of the presynaptic sites are well characterized, little is known about how they are assembled into a functional synapse. the Using the marker strain expressing GFP-tagged active zone (AZ) protein, CLA-1, and mCherry-tagged synaptic vesicle (SV)-associated protein, RAB-3, 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 wild type animals, CLA-1 is localized as a single punctum at the tip of the synaptic varicosity labeled with RAB-3. In the *unc-43(n498n1186)* loss-of-function mutant, we observed an increased number of CLA-1 puncta with weaker fluorescence intensity, which are often associated with diffuse localization of RAB-3, suggesting that *unc-43* is required for the proper presynaptic assembly. Consistently, *unc-43(n498)* gain-of-function mutants have “overdeveloped” synapses with increased CLA-1 and RAB-3 fluorescence intensity. To determine which presynaptic proteins’ localization is affected in *unc-43,* we are currently using the Native- and Tissue-Specific Fluorescence (NATF) strategy (He et al., 2019; Goudeau et al., 2021) to examine the localization of endogenous synaptic proteins including SYD-2, UNC-10, ELKS-1, and CLA-1.

To examine the functional conservation between UNC-43 and human CaMKII in presynaptic assembly, we generated a C. elegans strain in which endogenous *unc-43* locus was replaced with codon-optimized human CaMKIIA (hCaMKIIA) cDNA. We found that the *hCaMKIIA* animals exhibit wild type locomotion and have normal presynaptic structure, suggesting the functional conservation between UNC-43 and human CaMKIIA. We then introduced recessive (H477Y) and dominant (K291P) mutations that are found in the CaMKII gene from patients with intellectual disabilities (Chia et al., 2018; Onori et al., 2018). Indeed, these disease-associated mutations in hCaMKIIA resulted in presynaptic organization defects that are similar to those in the *unc-43(n498n1186)* and *unc-43(n498)* mutants. Our work reveals a conserved role of CaMKII in regulating the proper presynaptic assembly which we will use to further our understanding behind synaptopathies.

172 **A gene expression atlas of the entire L1 *C. elegans* nervous system** Seth R Taylor[1,2], Marc Hammarlund[3,4], Oliver Hobert[5], David M Miller[6,7], CeNGEN Consortium[3,5,61]Cell Biology and Physiology, Brigham Young University, [2]Vanderbilt University School of Medicine, [3]Genetics, Yale University School of Medicine, [4]Neuroscience, Yale University School of Medicine, [5]Biological Sciences, Columbia University, Howard Hughes Medical Institute, [6]Cell and Developmental Biology, Vanderbilt University School of Medicine, [7]Program in Neuroscience, Vanderbilt University School of Medicine

Nervous systems are actively remodeled during post-embryonic development as embryonic neurons mature and new neurons are integrated into functional circuits. To investigate the genetic programs that drive larval development of the *C. elegans* nervous system, we generated single-cell RNA sequencing profiles of ~100,000 cells, including >65,000 neurons from L1 hermaphrodite larvae. We detected 117 transcriptionally distinct neuron types including all known anatomically-defined neuron classes

present in mid-L1, several neuron classes born in the late L1, and new motor neuron subtypes. Comparison to our previously generated profiles of L4 neurons detected >20,000 instances of differential expression (DE) for >4,000 genes. Post-embryonically derived neurons (e.g., AVM, PVM) showed the highest number of DE genes as well as the most dramatic instances of DE. Most genes showed differential expression in a small number of neuron types (median = 2), indicating that larval maturation for individual neuron types involves largely distinct patterns of gene expression. The direction of differential expression (i.e., higher in L1 or higher in L4) was neuron-type dependent, as 25% of genes showed effects in opposite directions in different neuron types. Notably, genes involved in neuropeptide and synaptic signaling were highly represented among DE genes. In addition to post-mitotic neurons, we annotated clusters corresponding to neural progenitors from the Q, T, and P-cell post-embryonic lineages. We anticipate that this transcriptomic atlas will be a rich resource for investigations of genetic mechanisms of neuronal differentiation, development and function.

173 **Neuropeptide NLP-47 and its receptor GNRR-1 are important for forgetting of olfactory memory in *C. elegans*** Yuuki Onishi[1], Mary Arai[2], Jamine Hooi Min Teo[1], Tomohiro Kitazono[1], Takeshi Ishihara[21]Graduate School of Systems Life Sciences, Kyushu University, [2]Department of Biology, Faculty of Science, Kyushu University

Animals acquire and store information as memories that are required to regulate their behavior and decision-making. To mitigate undesirable effects of old information stored in their brain, they must forget some dispensable memories. However, molecular mechanisms in forgetting are still unclear. To investigate the mechanisms of forgetting, we use olfactory learning in *C. elegans* as a model. *C. elegans* is highly attracted to some odorants such as diacetyl, although, after prolonged exposure to odorants without food, the animals adapt to the odorants and show weak chemoattraction. The adapted animals can regain their chemoattraction after the cultivation on food for several hours, and this recovery can be considered as forgetting. Previously, our studies suggested that releasing of “forgetting signals” from AWC sensory neurons is important for forgetting of olfactory memory. However, the molecular basis of “forgetting signals” remains elusive.

Our previous study implies that neuropeptides might be responsible for forgetting signals. Therefore, to identify neuropeptides that serve as “forgetting signals” from AWC neurons, we searched for genes by using CeNGEN (*C. elegans* Neuronal Gene Expression Network, a dataset of single-cell RNA sequencing), and found 9 candidate of neuropeptide genes which are enriched in AWC. By using CRISPR-Cas9, we created these mutants and analyzed their forgetting phenotype. Among these candidates, *nlp-47* mutants showed forgetting defect, although they show normal chemoattraction and adaptation to diacetyl as wild type. Moreover, injection of wild-type genomic fragments could recover forgetting phenotype in the mutants. Cell-specific knockout of the neuropeptide gene suggests that releasing this neuropeptide from AWC is required for forgetting. Furthermore, our genetic analyses revealed that a neuropeptide receptor of this peptide, GNRR-1, which is conserved in human, is also important for forgetting.

Previously, Ca^{2+} imaging experiments on AWA neurons suggested that AWA store the sensory memory trace because the Ca^{2+} responses to diacetyl in AWA are positively correlated with behavioral changes after conditioning and recovery. To investigate whether the neuropeptide and receptor affect sensory memory trace in AWA, we examined AWA responses to diacetyl in naïve, after conditioning, and after the recovery, and found that the sensory memory trace in the mutants was remained even after recovery. This result is consistent with the behavioral change and suggests that the neuropeptide and receptor are involved in forgetting, presumably by degrading the memory trace in AWA.

Further analyses of these factors will reveal how memory forgetting are regulated by forgetting signals in animals.

174 **A pan-neuronal alternative splicing event triggers pan-neuronal gene transcription** Eduardo Leyva Diaz[1,2], Michael Cesar[2], Oliver Hobert[21]Instituto de Neurociencias, CSIC-UMH, [2]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

Gene expression programs in differentiating neurons can be subdivided into at least two types, those that control expression of neuron type-specific proteins and those that control expression of proteins that are shared by all cells in a nervous system, such as proteins involved in the synaptic vesicle cycle or in neuropeptide biogenesis. Pan-neuronal gene expression is controlled by members of the CUT homeobox gene family, including the pan-neuronally expressed *ceh-44*. We address here how the expression of *ceh-44* is directed to the nervous system. Our studies show that *ceh-44* pan-neuronal expression is triggered by a pan-neuronal RNA splicing factor, UNC-75, the *C. elegans* homolog of vertebrate CELF proteins. UNC-75 spatially specifies the production of an alternative, CEH-44 homeobox gene-encoding transcript from a ubiquitously expressed gene locus, which can also produce a conserved Golgi apparatus-localized Golgin protein, CONE-1 (“**C**ASP **o**f **ne**matodes”). During embryogenesis, before terminal tissue differentiation, the CONE-1/CEH-44 locus exclusively produces the Golgi-localized CONE-1 protein in all tissues, but upon the onset of postmitotic terminal differentiation of neurons, UNC-75 binds to the CONE-1/CEH-44 transcript to redirect the splicing machinery to now produce the alternative, CEH-44 CUT homeobox gene-encoding transcript, exclusively

in the nervous system. CEH-44 subsequently controls the expression of pan-neuronal effectors genes, such as proteins of the synaptic vesicle cycle. Hence, UNC-75-mediated alternative splicing not only directs pan-neuronal gene expression, but also excludes a phylogenetically deeply conserved Golgin from the nervous system, paralleling surprising temporal and spatial specificities of other Golgins that we describe here as well. Remarkably, the unusual combination of Golgin and homeobox gene production from a single locus is conserved in vertebrates as well. Our findings provide novel insights into how all cells in a nervous system acquire pan-neuronal identity features.

175 ***hrpr-1* rescues *smn-1*-related motoneurons degeneration by modulating *ret-1* splicing.** Pamela Santonicola[1,2], Federica La Rocca[1,3], Federica Cieri[1,4], Giada Onorato[1,3], Federica Rizzo[5], Mafalda Rizzuti[5], Giuseppina Zampi[1], Monica Nizzardo[5], Stefania Corti[5,6], Elia Di Schiavi[11]IBBR - CNR, [2]University of Molise, [3]University of Campania "L. Vanvitelli", [4]University of Naples "Federico II", [5]Foundation IRCCS Ca' Granda, [6]University of Milan

An efficient splicing of mRNA is required in all cells, but neurons seem to be more vulnerable to splicing perturbations. In fact, numerous neurodegenerative diseases are caused by splicing defects, such as Spinal Muscular Atrophy (SMA), Amyotrophic Lateral Sclerosis (ALS), and dementia. However, why neurons are more affected to splicing alterations and which step of the RNA processing is impaired in these diseases is still debated. SMA is caused by mutations in the Survival Motor Neuron (*Smn*) gene, which is involved in RNA metabolism and splicing. We previously demonstrated that genes differentially expressed or spliced in induced pluripotent cell-derived motor neurons (iPS-MNs) from SMA patients are enriched in the RNA motif 7. This motif is specifically recognized by hnRNP Q, a spliceosomal component physically interacting with SMN. We demonstrated that *hrpr-1,* the *hnRNP Q* homolog in *C. elegans*, is involved in MNs survival as well as *smn-1,* the *Smn* homolog. We confirmed their genetic interaction by nonallelic non-complementation and demonstrated that they exert their neuroprotective function specifically in MNs. In fact, *hrpr-1* overexpression in MNs rescues *smn-1* related neurodegeneration. Interestingly, comparing *hrpr-1* known targets in *C. elegans* and the alternatively spliced genes identified in SMA patients, we identified a new possible downstream target of the pathway, *ret-1*. *ret-1* is the only homolog in *C. elegans* of Reticulon genes, a family of transmembrane proteins involved in vesicle recycling and formation, and in neurite outgrowth. We confirm a possible involvement of *ret-1* in SMA, since we observed alteration in its transcript levels in *C. elegans*, SMA mice and patients. Moreover, we demonstrated that *ret-1* splicing pattern is altered when *smn-1* is depleted, and that *ret-1* is mediating the *hrpr-1* rescue of *smn-1*-related neurodegeneration. Finally, we demonstrated that *hrpr-1* and *smn-1* work together to guarantee the correct splicing of exon 5 of *ret-1* gene. Thus, we identified for the first time a neuroprotective role of *hrpr-1* and the involvement of *ret-1* in neurodegeneration.

176 **TIR-1/SARM1 inhibits axon regeneration and promotes axon degeneration** Lauren C O›Connor, Victoria L Czech, Brendan Philippon, Emily Norman, Alexandra B ByrneNeurobiology, UMass Chan Medical School

Severed axons must coordinate multiple complex processes to restore function to the injured nervous system. In neurons that are capable of repairing themselves, including many *C. elegans* neurons and the mammalian peripheral nervous system, the axon fragment that remains attached to the cell body regrows using a process called axon regeneration, and severed axon fragments distal to the injury are cleared. Identifying the molecular mechanisms that regulate both regeneration and destruction is important to understanding how multiple aspects of the injury response are coordinately regulated. In turn, such advances are important to restoring function to injured neurons in the mammalian central nervous system, which are incapable of repairing themselves. We recently found that TIR-1 functions within injured GABA motor axons to inhibit axon regeneration. This is an intriguing result because the *D. melanogaster* and mouse homologs of TIR-1, dSarm and SARM1, are critical regulators of the opposite response to injury, axon degeneration. Upon further examination, we found that TIR-1 also regulates degeneration of injured axon fragments that have been severed from both the cell body and neuromuscular junction. TIR-1 regulates the two seemingly opposite responses to injury, axon regeneration and degeneration, by interacting with distinct MAP kinase pathways on either side of the injury. TIR-1 inhibits axon regeneration by activating the NSY-1/ASK1 MAP kinase signaling cascade in the severed proximal axon fragment that remains attached to the cell body and promotes axon degeneration by interacting with the DLK-1/DLK/LZK mitogen-activated protein kinase signaling cascade in the severed distal fragment. The ability to inhibit axon regeneration is not unique to the TIR-1 homolog; we found that human SARM1 is also capable of inhibiting motor axon regeneration when expressed in *C. elegans* motor neurons. Our findings have led us to investigate 1) how do the specific MAPK pathways regulate each process? And 2) what molecular mechanisms determine whether TIR-1 functions with a specific MAPK pathway? We will present the data outlined above and our progress identifying upstream regulators of TIR-1 and downstream effectors of regeneration and degeneration. Identifying the mechanisms by which TIR-1 alters regeneration and degeneration will provide a greater understanding of the complex neuronal injury response and how it can be manipulated to drive the system toward repair.

177 **Metabolic mechanisms for suppression of axon degeneration** Hadas Dabas[1], Kevin De Leon[2], Marc Hammarlund[11]Genetics and Neurobiology, Yale, [2]Neurobiology, University of Puerto Rico

Axon degeneration is an evolutionarily conserved and tightly regulated process that is a hallmark of neurodegenerative diseases. Recent research indicates that mitochondrial function and localization are key regulators of axon degeneration, yet driving mechanisms remain elusive. Understanding the fundamental biology of axon degeneration can aid in identifying targets to combat neurodegenerative disease.

C. elegans mitochondria-trafficking-mutants (MTMs) such as *ric-7, unc-116*, and *mtx-2;miro-1* have no axonal mitochondria. Lack of mitochondria results in spontaneous axon degeneration[1,2]. Thus, MTMs are a powerful model for studying axon degeneration linked to mitochondrial dynamics.

We discovered that inhibiting glycolysis in MTMs strongly suppresses axon degeneration. We tested multiple methods of inhibiting glycolysis, including drugs and genetic mutants; the two MTMs *ric-7* and *mtx-2;miro-1*; and both PVQ and DVC neurons. In all cases, inhibiting glycolysis suppressed degeneration. Inhibiting glycolysis is expected to reduce ATP levels. Thus, our findings suggest that axon degeneration in MTMs is not due to low ATP levels, since degeneration can be suppressed by further lowering ATP.

What then are the molecular mechanisms involved in MTM axon degeneration? We hypothesize that axonal lactate accumulation drives axon degeneration. Pyruvate, the end product of glycolysis, is normally taken up by mitochondria and used to power the TCA cycle. Alternatively, pyruvate can be converted to lactate by lactate dehydrogenase (LDH). Thus, in the absence of mitochondria, lactate may accumulate in axons and drive degeneration; blocking glycolysis in this context lowers lactate levels and thus suppresses degeneration. Accordingly, mutation or degron-mediated knockdown of LDH suppresses axon degeneration in MTMs.

To further test this model, we apply a lactate/pyruvate FRET sensor and a pH sensor to measure the axonal environment in MTM mutants. We use microbial enzymes with catalytic activities, not found in metazoans, to alter lactate levels in MTM neurons. Furthermore, we developed an optogenetic assay to measure function of the DVC neuron, revealing the relationship between function, metabolism, and degeneration. Together, these experiments reveal molecular mechanisms of axon degeneration, furthering our understanding of mitochondria and metabolism in health and disease.

[1]Rawson et al. 2014 doi:10.1016/j.cub.2014.02.025
[2]Zhao et al. 2021 doi:10.1038/s41467-020-20346-2

178 **Neural mechanisms of oxygen sensing in a skin-penetrating nematode** Breanna Walsh[1,2,3], Elissa A. Hallem[1,41]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, [2]Molecular Biology Interdepartmental PhD Program, University of California, Los Angeles, [3]UCLA-Caltech Medical Scientist Training Program, University of California, Los Angeles, [4]Molecular Biology Institute, University of California, Los Angeles

Soil-transmitted helminths (STHs) infect at least 24% of the global population, with resultant illness disproportionately impacting under-resourced communities. *Strongyloides stercoralis* is a skin-penetrating, human-infective STH that infects roughly 600 million individuals. *S. stercoralis* infective third-stage larvae (iL3s) use external sensory cues (*e.g.*, host body heat, host-emitted odorants) for environmental navigation, active host seeking, and resumption of development to intra-host stages. In transitioning from the free-living to intra-host environment, *S. stercoralis* iL3s encounter oxygen (O_2) concentrations ranging from 21% (*i.e.,* atmospheric levels) at the soil surface to ~5% in host tissues. We posit that *S. stercoralis* iL3s likely use O_2 as a sensory cue that enables parasitic behaviors and development; however, O_2 sensing remains unstudied in *S. stercoralis* and all other parasitic nematodes. The O_2-sensing behaviors of *C. elegans* dauers (*i.e.,* the developmentally arrested third-stage larvae most analogous to *S. stercoralis* iL3s) also remain uncharacterized.

We found that *S. stercoralis* iL3s demonstrate robust changes in locomotion when exposed to acute shifts in O_2 concentration. When exposed to 7% O_2, iL3s exhibit a gradual slowing response that is reversible when worms return to 21% O_2. These responses are the first O_2-evoked behaviors to be described in any parasitic nematode. We also found that *C. elegans* Hawaii dauers sense and respond to changes in O_2 concentration, albeit with motile behaviors that are distinct from those in *S. stercoralis* iL3s.

To uncover the molecular and neuronal bases of O_2 sensing in *S. stercoralis*, we used phylogenetic analysis to identify four candidate O_2 sensors, each of which are protein-level homologs to the O_2-sensing soluble guanylate cyclases (sGCs) found in *C. elegans*. Using transcriptional reporters, we found that at least three of the *S. stercoralis* sGCs are expressed in neurons that bear anatomic and morphologic similarity to known *C. elegans* O_2-sensing neurons. In *S. stercoralis,* chemogenetic silencing of putative O_2-sensing neurons resulted in blunted O_2-evoked motile behaviors. We are now examining the encoding properties of these neurons by calcium imaging and comparing the neuronal response properties to those of O_2-sensing neurons in *C. elegans* dauers. Our results will illuminate how species-specific variation in O_2 sensation can facilitate behaviors that support parasitism.

179 **Hierarchical regulation of functionally antagonistic neuropeptides expressed in a single modulatory neuron type control locomotion of *C. elegans*** Ichiro Aoki[1], Shripriya Bhat[2], Alexander Gottschalk[21]Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, [2]Goethe University Frankfurt

Communication among cells via neuropeptides is crucial for proper function of the nervous system. Across diverse species, many neurons express multiple neuropeptides and small molecule transmitters, raising the question whether these neurons simply co-release cocktails of transmitters, or some of those neurons may release transmitters separately and specifically.

To address this question, we used the AVK inter/modulatory neurons as a model system that express multiple neuropeptides including FLP-1 and others, which affect locomotion and arousal[1,2]. To examine the role of AVK on locomotion in detail, we optogenetically activated AVK and found that it accelerated locomotion speed despite the fact that FLP-1, the most abundantly expressed neuropeptide in AVK, suppresses locomotion[3,4]. We therefore aimed to identify the factor that transmits a locomotion accelerating signal from AVK and screened neuropeptides expressed in these cells, since no small molecule transmitter is known to be expressed in AVK. We found that NLP-10 and its receptor NPR-35[5] are required for acceleration induced by AVK-photoactivation. Remarkably, the effect of AVK-derived NLP-10 was completely abolished in *npr-35* mutants, suggesting NPR-35 is the sole receptor for NLP-10 at least in this context. Secretion of NLP-10 from AVK was decreased in the absence of FLP-1 in AVK, consistent with antagonizing roles of these neuropeptides on locomotion speed. This result suggests either that FLP-1 suppresses NLP-10 release through autocrine or that both peptides compete for the release machinery. Interestingly, FLP-1 and NLP-10 were differentially packaged into dense core vesicles, suggesting that trafficking and/or release of these peptides are differentially regulated. We are now identifying the downstream neural circuit by performing cell-specific rescue experiments for *npr-35* mutants to clarify how AVK-derived FLP-1 and NLP-10 antagonistically regulate locomotion.

Intriguingly, NLP-10 and NPR-35 were required for acceleration upon blue light illumination, especially after repeated illumination, suggesting NLP-10-NPR-35 signaling actively suppresses habituation to noxious stimuli.

Taken together, our study showed that two hierarchically regulated neuropeptides expressed in the same cell, antagonistically regulate locomotion.

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180 **Identifying the aging intestine secretome and its role in lifespan regulation** Jason W Miklas[1], Nicole R Haseley[2], Anne Brunet[11]Genetics, Stanford University, [2]Stanford University

Organs communicate to maintain homeostasis. Understanding the mechanisms by which organs communicate and how this is affected during aging could provide new avenues for therapeutic interventions to counter aging and disease. Secreted proteins have the exciting potential to be critical in organ-to-organ communication and potentially regulate lifespan. Yet, it is not clear how the secreted protein landscape changes with age and how this contributes to organismal decline. I am particularly interested in how the intestinal 'secretome' changes with age because the intestine has a unique multifaceted role in metabolism and immunity, interfaces with the microbiome, and can act as a signal generator to distal tissues. I have developed a tissue and compartment specific protein labeling tool by localizing the biotinylating protein TurboID to the intestine endoplasmic reticulum of *C. elegans*. Using quantitative proteomics, I characterize the intestine secretome – the ensemble of secreted proteins and secretion machinery proteins – in young and old worms and identified 71 proteins that changed with age. To systematically understand how the intestine secretome becomes disrupted with age, I generated transcriptional and translational reporters of 5 of these proteins along the secretion pathways. Notably, I identified a secreted protein whose secretion pattern change with age. To determine the impact of intestine secretome proteins on lifespan, I performed lifespan assays for these 5 intestine secretome proteins that either significantly increased or decreased with age using RNAi. I found that two of them, when knocked-down, significantly extended lifespan. I also overexpressed these genes in the intestine and assessed lifespan. I found that intestine specific overexpression of one of these genes led to a significant decrease in lifespan. Together, my experiments uncover new proteins along the secretion machinery, included secreted proteins. My results also implicate the intestine secretion machinery and secreted proteins as key players in regulating lifespan, which could provide new strategies to counter aging and disease.

181 **Ribosome rescue factor PELO-1/PELOTA prevents premature aging by enhancing mRNA quality and autophagy in *C. elegans* and mammals** Jongsun Lee[1,1], Bora Lee[2,2], Hye-in Lee[3,3], Eun Ji E. Kim[1], Seokjin Ham[1], Eunah Kim[1], Hae-Eun H. Park[1], Sieun S. Kim[1], Yoonji Jung[1], Hyunwoo C. Kwon[1], Seokjun G. Ha[1], Jinsoo Seo[3], Kwang Pyo Lee[2], Seung-Jae V. Lee[11]Biological Sciences, Korea Advanced Institute of Science and Technology, [2]Korea Research Institute of Bioscience and Biotechnology, [3]Brain Sciences, Daegu Gyeongbuk Institute of Science and Technology

Ribosome-associated quality control (RQC) is an essential biological process for maintaining the proper quality of ribosomes, functioning in conjunction with nonstop decay (NSD) and no-go decay (NGD) mRNA surveillance pathways. Defects in RQC and the mRNA surveillance pathways impair cellular homeostasis, but their role in aging remains largely unknown. Here, we show that the RQC system that enhances both mRNA and protein quality promotes longevity and delays aging in *C. elegans* and mammals. Through a genetic screen targeting 27 conserved RQC and mRNA surveillance factors, we found that genetic inhibition of PELO-1/Pelota, the ribosome rescue factor, and SKIH-2/SKIV2L, a component of 3'-to-5' exonuclease complex, robustly decreased lifespan. We also found that *pelo-1(cc2849)* and *skih-2(cc2854)* mutations exacerbated age-dependent motility declines and Alzheimer's disease (AD) model-associated paralysis. We showed that PELO-1 and SKIH-2 were downregulated in aged animals, which we showed to display reduced RQC, NSD and NGD activities. We found that *pelo-1* and *skih-2* were required for the longevity of various established mutants, including insulin/IGF-1 receptor *daf-2(e1370)* mutants, which exhibited enhanced mRNA surveillance. Thus, PELO-1 and SKIH-2 appear to be essential for longevity and healthspan. Next, we sought to determine how RQC and the mRNA surveillance regulated by *pelo-1* and *skih-2* affected aging. Our RNA sequencing analysis indicated that genes that were differentially expressed by *pelo-1; skih-2* double mutations in *daf-2* mutants significantly overlapped with those by mutations in *hlh-30*/*TFEB*, the master regulator of autophagy. In addition, *hlh-30(tm1978)* mutations did not further decrease the short lifespan of *daf-2 pelo-1; skih-2* mutants. Further, by using HLH-30::GFP and GFP::LGG-1, we showed that the *pelo-1* and *skih-2* mutations reduced autophagy activity in *daf-2* mutants. Thus, PELO-1 and SKIH-2 appear to promote longevity via enhancing autophagy. As the role of Pelota in RQC and the mRNA surveillance is conserved in eukaryotes, we next focused on determining the function of Pelota/PELOTA in aging and age-associated diseases using mammalian systems. We found that *PELOTA* knockdown accelerated senescence in cultured human IMR90 cells. In addition, muscle-specific *Pelota* knockout caused sarcopenia and substantially reduced lifespan in mice. Further, *PELOTA* knockout exacerbated the pathogenesis in human iPSC-derived AD model neurons. Lastly, we showed that the *Pelota*/*PELOTA* knockout decreased autophagy, by assessing autophagy markers, LC3 and p62, in mice and the human iPSC-derived AD neurons. Thus, PELO-1/Pelota is critical for longevity and the prevention of age-associated diseases by enhancing autophagy-mediated proteostasis as well as RQC and mRNA surveillance, conserved from *C. elegans* to mammals.

182 **Maternal insulin signaling to germ cells regulates adaptive changes in offspring metabolism via a mitochondrial ETC dependent mechanism** Kim Nguyen, Jason Cooper, Nick BurtonVan Andel Institute

Growing evidence from model genetic organisms (*C. elegans, D. melanogaster*) indicates that insulin signaling to oocytes regulates offspring metabolism across diverse taxa. For example, we previously found that reduced maternal insulin signaling to germ cells in *C. elegans* promotes a >10-fold increase in offspring survival in response to osmotic stress. This maternally regulated adaptation is mediated, at least in part, by increasing glycerol production in offspring. How reduced insulin signaling to oocytes might promote such adaptive changes in offspring metabolism, however, is unknown. Recently, we found that the effects of maternal insulin signaling to germ cells on offspring response to osmotic stress in *C. elegans* are transmitted via a mechanism that depends on proper mitochondrial electron transport chain (ETC) function. Furthermore, we found that the effects of reduced insulin signaling to germ cells on offspring response to osmotic stress are lost in many different mitochondrial ETC mutants (*nuo-6, nduf-7, gas-1, mev-1, isp-1*). To determine how mitochondrial ETC mutations disrupt the effects of maternal insulin signaling on offspring we performed a chemical mutagenesis screen for suppressors of *isp-1* mutants' failure to adapt to osmotic stress. From this screen we found that the loss of the AMP-kinase subunit AAK-2 restores the ability of *isp-1* mutants to adapt to osmotic stress. By combining these genetic findings with broad metabolomics and transcriptomics approaches, we propose a model in which reduced insulin signaling to oocytes promotes a remodeling of oocyte mitochondria (as has been reported in flies and frogs – Sieber *et al., Cell* 2016). This remodeling in in oocyte mitochondria in turn alters long-term offspring metabolism in such a way that promotes increased glycerol production in larvae and increased resistance to osmotic stress by altering signaling via AAK-2. If true, these findings suggest that oocyte mitochondria might transmit environmental information from mothers to offspring independently of any change in offspring DNA sequence (mtDNA or genomic DNA). Furthermore, similar observations of insulin regulated mitochondrial remodeling in fly and frog oocytes suggest that such a phenomenon might be broadly conserved throughout evolution and potentially explain some of the epidemiological links between maternal environments and offspring to susceptibility to various metabolic pathologies in humans.

183 **Nutrient deprivation induces a tissue-specific, reversible, large-scale chromatin reorganization *in C. elegans*** Nada Al-Refaie[1,2], Johanna Hornung[1], Francesco Padovani[1], Francesca Binando[1], Elif Sarinay Cenik[3], Kurt Schmoller[1], Daphne Selvaggia Cabianca[11]Institute of Functional Epigenetics, HelmholtzZentrum Muenchen, [2]Faculty of Medicine, LMU, [3]University of Texas at Austin

Lack of nutrients is a major environmental input in biology with feeding/fasting cycles occurring in virtually all animals. Epigenetic mechanisms link the environment to chromatin regulation. Yet, whether and how, fasting impacts on chromatin organization in a multicellular organism remains unknown.

Here, we used *C. elegans* to investigate the effects of fasting on 3D chromatin architecture within tissues of intact animals by live microscopy. Strikingly, we found that during fasting chromatin within all intestinal cells undergoes a large-scale spatial reorganization which is rapidly reversed by refeeding, revealing a dynamic process.

Treating fed animals with Actinomycin D, induces a fasting-like chromatin redistribution, suggesting that a transcriptional shutdown might promote the spatial genome reorganization observed in absence of nutrients. To address the specific role of the three different RNA polymerases, we used the auxin-inducible degradation system and found that the degradation of an RNA pol I-specific subunit is sufficient to i) trigger a fasting-like chromatin architecture in fed animals and ii) impede the restoration of a fed-like 3D genome organization when fasted animals are refed. Moreover, nucleolar size is drastically reduced in fasted intestinal cells indicating that rRNA synthesis is diminished. Thus, RNA pol I activity is central to the chromatin reorganization induced by lack of nutrients.

During fasting, the genome of intestinal cells becomes enriched at the nuclear periphery and around the nucleolus, forming “chromatin rings” readily visible in confocal images. To gain insights into a potential function of this reorganization, we used a fluorescence-based tool to monitor both the activity and positioning of an active allele during fasting, at the single-cell level. Remarkably, alleles localizing within either one of the two “chromatin rings” display decreased expression compared to those positioned away, suggesting that the “chromatin rings” induced by fasting are repressive environments.

Altogether, this work revealed that a physiological environmental stimulus, such as fasting, induces a large-scale, tissue-specific reorganization of chromatin architecture and uncovered an unprecedented role of RNA Pol I in shaping the 3D genome organization of a metabolic tissue.

184 **Multi-species nematode screening uncovers a new class of broad-spectrum anthelmintic compounds** Hala Zahreddine Fahs[1], Fathima Refai[2], Suma Gopinadhan[2], Gennaro Esposito[2], Patricia Cipriani[1], Yasmine Moussa[2], Stefan Kremb[2], Xin Xie[2], Yanthe Pearson[2], Rick Maizels[3], Antony Page[3], Fabio Piano[1,2], Kristin C Gunsalus[1,21]NYU, [2]NYU Abu Dhabi, [3]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 urgently needed, as resistance to existing drugs is emerging. Using the NYUAD HTS platform, we screened 35,000 compounds for broad anthelmintic properties while being non-toxic to human cells. The screen identified most known anthelmintics and numerous compounds with no previously reported anthelmintic activity. Among these, four related natural compounds caused dose-dependent lethality in Caenorhabditis elegans and Pristionchus pacificus across all developmental stages, including dauer and embryos, while being relatively well tolerated in mammalian U2OS cells. These molecules also caused mortality in direct testing on three veterinary parasitic nematode species: the multi-drug resistant Haemonchus contortus UGA strain (ruminants), Teladorsagia circumcincta (sheep and goat) and Heligmosomoides polygyrus (rodents). In vivo preclinical trials in mice infected by Heligmosomoides polygyrus helminths further showed that these compounds cause a consistent and significant reduction in H. polygyrus egg laying and adult worm load. In-depth phenotypic characterization in C. elegans revealed abnormal lipid accumulation, mitochondrial defects, reduced oxygen consumption, diminished ATP levels, and increased reactive oxygen species. Together with genetic and pharmacological perturbations, these results point to lipid metabolism as the key target pathway of these novel anthelmintic compounds.

185A **High-throughput screening of fluorescent *Caenorhabditis elegans* using the COPAS VISION large particle imaging flow cytometer** Giuliano Ferrero, Francis Smet, Tom Mullins, Mariya Lomakina, Yifei WangUnion Biometrica

Union Biometrica’s large particle flow cytometers enable the analysis and gentle dispensing of large objects, such as *C.elegans,* zebrafish and insect larvae, cell clusters and seeds. The COPAS VISION instrument pushes the boundaries of flow cytometry by adding a brightfield camera that captures images of the samples as they pass through the flow cell. Sorting decisions of the objects are made based on measurements of relative size, optical density and fluorescence, as well as localized fluorescence as determined by the Profiler. The collected images and flow cytometry measurements are synchronized so that objects dispensed

to wells of multiwell plates can be traced back to their corresponding image. The FlowPilot software, designed to acquire cytometry data, boasts a dedicated image processing suite where the profiler graph can be superimposed on the acquired images to match the signal with the corresponding areas of the sample. We hereby use a COPAS VISION to image *C.elegans* at different stages and to assess the expression of mCherry in the pharynx. The images we obtained helped determining how the orientation and conformation of the sample in the flow stream correlate with the optical density and fluorescence profiles, confirming the benefits of adding imaging features to the cytometer.

186A **Analyses of mechanisms of ALLO-1 targeting to the paternal organelles in embryos** Takuya Norizuki[1], Taeko Sasaki[2], Yasuharu Kushida[2], Nobuo N. Noda[3], Ken Sato[2], Miyuki Sato[21]Institute for Molecular and Cellular Regulation, Gunma University, [2]Gunma University, [3]Hokkaido University

Autophagy is a conserved catabolic system for lysosomal degradation of cytoplasmic components, and is involved in various developmental and physiological processes. We previously reported that autophagy is required for degradation of sperm-derived mitochondria and membranous organelles in *Caenorhabditis elegans* embryos (Sato and Sato, 2011, *Science*), and subsequently, similar processes were also reported in mammals and Drosophila. Our further analyses identified ALLO-1 as an autophagy adaptor which recruits autophagy machinery components to paternal organelles in *C. elegans* embryos (Sato et al., 2018, *Nat. Cell Biol.*). However, it still remains unclear how oocyte-derived ALLO-1 is recruited to paternal organelles after fertilization. In this presentation, we will present our latest data on regulation of ALLO-1 targeting to the paternal organelles in embryos.

187A **Ciliopathy Disease Modelling in *C. elegans*: using CRISPR to interpret patient variants and characterise molecular mechanisms of Joubert Syndrome** Karen I Lange, Emilia Filipczak, Sofia Tsiropoulou, Oliver BlacqueUniversity College Dublin

Primary cilia are microtubule based organelles on the cell surface that function as sensory and signalling antennae. The transition zone is a distinct region at the base of the cilia that acts as a membrane and cytosolic diffusion barrier that is important to maintain the unique composition of the organelle. Defects in cilia structure/function are associated with diseases known as ciliopathies. One such condition, Joubert syndrome, is a rare genetic disorder characterised by a midbrain-hindbrain malformation and a wide range of associated symptoms (developmental delay, breathing abnormalities, liver and kidney dysfunction, and retinal degeneration). More than 40 Joubert syndrome genes are known, with many encoding proteins that localise to the transition zone at the base of the cilium. Many patient variants, especially missense mutations, are classified as variants of uncertain significance (VUS) due to insufficient evidence to conclude on their pathogenic or benign nature. A VUS classification is not clinically actionable towards disease molecular diagnosis, management, treatment and counselling. Modelling and characterising VUS in *C. elegans* can provide evidence about their pathogenicity. This project is using CRISPR-Cas9 genome editing to model missense and truncating patient variants (pathogenic and VUS) in four Joubert syndrome genes: *RPGRIP1L* (*mks-5*), *CEP290* (*cep-290*), *CC2D2A* (*mks-6*), and *TMEM67* (*mks-3*). Endogenous worm genes are tagged with GFP and the patient variants are engineered into conserved positions. Quantitative assays of cilia structure and function (dye filling, chemotaxis, osmotic avoidance, roaming, protein localisation) are used to determine the effect of the mutations relative to wild type and null mutant controls. Variants that affect gene function (ie. pathogenic variants) are further characterised with electron microscopy to investigate transition zone ultrastructure and biotin proximity labelling (TurboID) to determine how the mutations affect the molecular composition of the transition zone. This project is part of a larger collaboration (NDCil) to characterise these same variants in other animal and cell-based systems (zebrafish, mice, patient derived stem cells). The overall goal of the project is to provide in vivo evidence for interpreting and reclassifying patient VUS, and establish allelic series of worm strains for elucidating Joubert Syndrome disease mechanisms.

188A **Dynamics of oocyte-surface proteins during and after fertilization** Sugiura Kenta, Sato KenInstitute for Molecular and Cellular Regulation, Gunma University

Fertilization is an essential phenomenon for sexual reproductive organisms to produce offspring and requires membrane fusion between a male and a female gamete. In *C. elegans*, a subset of maternal Plasma Membrane (PM) proteins are selectively internalized from the PM and degraded in the lysosomes after fertilization. For example, CAV-1-GFP, which localizes to cortical granules in oocytes, is immediately targeted to the PM after fusion and then degraded during oocyte-to-embryo transition.

EGG complex components including EGG-1–5, CHS-1 and MBK-2 also undergo degradation at zygotes. Transmembrane proteins EGG-1, EGG-2 and CHS-1 localize on the oocyte PM, and the other factors peripherally associate with the cortex region with the PM proteins. Time-lapse imaging for fluorescent protein-tagged-EGG complex components indicated that the proteins were evenly distributed on the oocyte PM, and then formed to punctate structures approximately 15 min after fertilization. While the degradation of EGG complex proteins were also observed under fertilization defective mutants, the punctation and internalization of EGG-1 were not induced in infertile mutants, suggesting that the mechanism of EGG-1 degradation is distinct from the other components and depends on fertility.

Live imaging analyses revealed another aspect of the dynamics of the EGG complex proteins at the timing of the sperm-oocyte fusion. The signal intensity of the EGG complex components at the sperm contact region were instantly decreased, then recovered after a few ten seconds. We referred such regions to as a Fertilization Zone (FZ), where the signals of the maternal membrane proteins localized to the oocyte PM are transiently lost due to sperm contact/fusion. We also found that the signals of actin filaments and vitelline layer proteins, which interlines and covers the oocyte PM, respectively, as well as the FZ of the EGG complex proteins, transiently disappears and recovers during fertilization.

GFP-tagged PH (PLCd1), which peripherally associates with the oocyte PM, also showed the FZ. Fluorescent signal decreasing durations showed statistical differences; the FZ duration of EGG components maintained longer time than that of PH (PLCd1), suggesting that the conditions of the FZ are differed by proteins characteristics. We are now trying to investigate the physiological significance and molecular mechanisms underlying the FZ formation in fertilization in detail.

189A **Identification of polycystin interactors within ciliary extracellular vesicles using proximity labeling in *Caenorhabditis elegans* Topics: Cell Biology / Intracellular Trafficking and Organelles** Inna Nikonorova[1], Elizabeth desRanleu[1], Katherine Jacobs[1], Joshua Saul[2], Jonathon Walsh[2], Juan Wang[1], Maureen Barr[11]Genetics, Rutgers University, [2]Rutgers University

Intercellular communication via extracellular vesicles (EVs) implies existence of mechanisms for precise packaging and targeting of cell-specific signaling cargo. Identification of cargo composition of individual EV subtypes is integral to understanding their bioactivity and one of the major challenges of the EV field.

In *C. elegans*, signaling ciliary EVs are released from male-specific ciliated neurons and transferred to the vulva of a mating partner, suggesting a role in inter-organismal communication. We recently identified the proteome of ciliary EVs released by the animal into the environment. Here we present a much-improved pipeline for dissection of the proteome of individual EV subtypes using a proximity-labeling approach.

We targeted a proximity-labeling enzyme TurboID to the evolutionarily conserved EV cargo polycystin PKD-2::GFP using an anti-GFP nanobody domain. Targeted EVs were enriched using buoyant density centrifugation, followed by pulldown of biotinylated proteins. Mass spectrometry analysis revealed 20 candidate interactors of PKD-2, as opposed to 2,888 EV cargo of the whole EV proteome. For validation, we generated CRISPR knock-in fluorescent reporters for the top 8 hits and analyzed their trafficking to PKD-2-carrying ciliary EVs.

Results: We discovered that PKD-2 proximity-interactors comprise two categories: (i) a genus-specific set of transmembrane adhesion proteins and (ii) an evolutionarily conserved set of soluble proteins with signaling function. All analyzed proximity-interactors colocalize with PKD-2 in cilia and ciliary EVs. The adhesion proteins are expressed and shed in ciliary EVs in non-overlapping PKD-2-expressing ciliated sensory neurons, suggesting a potential role in EV targeting. In contrast, evolutionarily conserved soluble cargo are expressed in all PKD-2 expressing neurons and depend on the polycystins LOV-1 and PKD-2 for their loading to EVs, suggesting a role in EV signaling. We are currently testing these hypotheses.

Conclusion: Coupling density equilibration with pulldown of biotinylated proteins resulted in at least 10,000-fold specific enrichment for interactors of the targeted EV cargo with virtually no false-positive hits. This methodology may be applied systematically to many EV cargo to break the code of ciliary EV cargo sorting and combinatorial composition.

190A **Mitochondrial morphology and inheritance during asymmetric cell division** Jens Van Eeckhoven, Ioannis Segos, Barbara ConradtCell and Developmental Biology, University College London

Mitochondria are essential to eukaryote cells. These power plants of eukaryote cells cannot be synthesised *de novo* and therefore need to be transferred from mother cell to daughter cells. During symmetric cell divisions, they typically distribute evenly among daughter cells. In asymmetrically dividing cells however, they can distribute non-randomly and this has been proposed to contribute to or cause changes in cell fate. How mitochondria impact cell fate in this context is unknown. In *C. elegans*' Q lineage, asymmetric division can generate an apoptotic cell death. Using the division of QL.p as a model, we studied mitochondrial distribution and morphology at super resolution (see Segos *et al.*'s poster). Principal component analysis (PCA) of six mitochondrial shape and size parameters allowed us to reduce the data to three dimensions retaining ~90% of all variance. The first three principal components (PCs) mainly contained size variation (PC1), variation in ellipticity (PC2), and variation in sphericity and surface/volume ratio (PC3), respectively. Using this approach, we found that the apoptotic daughter cell QL.pp receives fewer mitochondria during division and that these mitochondria are on average smaller (PC1) and more rounded (PC3). When we tested this in symmetric QL.p divisions (*pig-1 /MELK* mutant), the biased segregation between daughter cells disappeared. We previously showed that mitochondria in apoptotic cells are fragmented (Jagasia *et al*, 2005). Our results suggest that there already is a bias in their segregation during cell division. To further investigate the role of mitochondrial morphology in this

process, we assayed the survival of QL.pp in mitochondrial fission (*fzo-1*) and fusion (*drp-1*) mutants. *fzo-1* mutants have small and round mitochondria exclusively, whereas *drp-1* mutants have long tubular mitochondria. Segregation of mitochondria during asymmetric QL.p division was disrupted in these mutants, with both mutants showing an increased variance in the mitochondrial material inherited by the daughter cells. Additionally, QL.pp survived ~8% of the time in both of these mutants. Overall, our results indicate that the morphology and number of mitochondria inherited by QL.pp assume a role in QL.pp's ability to undergo apoptosis.

191A **Extracellular matrix regulates polycystin localization and extracellular vesicle release from *C. elegans* sensory cilia** Katherine C Jacobs[1], Deanna M De Vore[2], Karla M Knobel[3], Ken C Q Nguyen[4], David H Hall[4], Maureen M Barr[11]Department of Genetics and Human Genetics Institute of New Jersey, Rutgers University, [2]Diane C. Lobosco STEM Academy, [3]Waisman Center, University of Wisconsin-Madison, [4]Albert Einstein College of Medicine

The primary cilium is a signaling organelle on many cell types that senses external signals via transmembrane receptors and ion channels. Cilia also send signals by releasing extracellular vesicles (EVs). Ciliary defects underlie a class of human diseases known as ciliopathies. Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a ciliopathy caused by mutations in the transmembrane polycystin proteins, PC1 and PC2, which localize to renal primary cilia and are cargoes of urinary EVs. Despite decades of research, the function of polycystins and regulation of their localization remain enigmatic. In *C. elegans*, the polycystin homologues LOV-1 and PKD-2 localize to the sensory cilia of male-specific neurons and are cargoes of cilia-derived EVs, making *C. elegans* an excellent model for the study of polycystin localization and function as well as the fundamental biology of EVs.

We have previously found that *C. elegans* mutants for a putative extracellular matrix (ECM) gene *mec-9* display PKD-2 overaccumulation in cilia and at the ciliary base. We hypothesize that ECM plays a role in regulating polycystin localization and function. Additionally, *mec-9* mutants have increased levels of EV release, raising the possibility that ECM affects ciliary EV biogenesis. Investigation of how *mec-9* affects polycystin localization and EV release will be discussed. A hallmark of ADPKD is renal fibrosis caused by dysregulation of ECM, and this work will shed light on the relationships between ECM, polycystins and EVs.

192A **A split-GFP based biosensor for the detection of mature CED-3 caspase *in vivo*** Madiha J. Ghani[1], Hai Wei[2], Eric J. Lambie[1,2], Barbara Conradt[1,21]Cell and Developmental Biology, University College London, [2]Faculty of Biology, LMU Munich

Caspases are **c**ysteine-dependent **asp**artate-directed proteases that are key downstream effectors of the apoptosis pathway in animals as diverse as *C. elegans* and mammals. Caspases are synthesized as essentially inactive 'pro-caspases', which can be found in most if not all cells. In cells programmed to die, apoptosomes are assembled, and this enables the cleavage and maturation of pro-caspases to the fully matured and active proteases. Each mature caspase is generated from two pro-caspase molecules and composed of two small and two large subunits. Once caspase activity in a cell has reached a certain 'lethal' threshold, apoptotic cell death is triggered.

Using indirect assays, our group has previously obtained evidence that a low level of activity of the *C. elegans* caspase CED-3 is already present in mothers of cells programmed to die and that this low level of CED-3 caspase activity is asymmetrically inherited by the daughter that is programmed to die. However, because of the lack of a caspase sensor, we have so far been unable to directly visualise and quantify mature CED-3 caspase in real time *in vivo*. To address this, our current work focuses on designing a biosensor for quantitative and real-time measurements of active CED-3 caspase *in vivo*. We will utilize a split-GFP (GFP1-10 and GFP11) approach to monitor the assembly of the mature CED-3 caspase rather than CED-3 caspase activity *per se*, which will allow us to obtain not only single cell but also subcellular resolution. Using CRISPR/Cas12a mediated genome editing, the endogenous *ced-3* locus will be edited to generate a CED-3 protein tagged at its C-terminus with a 15 amino acid flexible linker and a codon-optimised GFP11 fragment. The complementary GFP1-10 fragment will be inserted into a *ced-3* transgene, which will be integrated into the genome using miniMos technology. We expect that the maturation of CED-3 caspase will result in the assembly of functional GFP, capable of emitting fluorescent light. Once the senor has been established and validated, our objective is to investigate the maturation of CED-3 caspase in cell death lineages and the impact of cell size on maturation. This sensor will also help to determine the subcellular localization of mature CED-3 caspase and to study CED-3's non-apoptotic roles in *C. elegans* development.

193A **Linker cell-type death (LCD) – a physiological model for nuclear and chromatin aberrations** Olya Yarychkivska, Yun Lu, Shai ShahamThe Rockefeller University

Changes in nuclear morphology and chromatin organization are commonly observed in physiological and pathological conditions such as developmental cell death, aging, progeria, and aggressive cancers. However, the precise consequences, implications and molecular nature of these changes for disease progression are not well understood. Aspects of these nuclear changes are also seen during the demise of the *C. elegans* linker cell. This cell is a sex-specific migrating leader cell that transitions from having an

oval nucleus to one with a deformed nuclear envelope as it undergoes non-apoptotic, caspase-independent programmed cell death (LCD, Linker Cell-type Death). To follow dynamic changes in nuclear morphology and chromatin during linker cell death, we used endogenously tagged fluorescent Lamin protein to show that the nuclear lamina acquires unusual ring formations that precede onset of visible NE deformations. The cell then undergoes stereotypic events of budding, splitting (small cytoplasmic fragment buds off), and engulfment resulting in a refractile cell morphology. Using animals staged at different times, as well as long-term live imaging of individual animals, we discovered that the nuclear lamina dissociates as the LC splits. Simultaneously, linker histone H1 and nucleosome-associated histone H2B staining transitions from nuclear puncta to a cell-wide diffuse distribution. Surprisingly, following budding, the lamina fully reassembles with the unusual ring formations, resembling changes observed in metastatic cancer cells, and histones are re-incorporated into chromatin puncta. EM studies support our observations revealing nuclear envelope defects, including inner nuclear membrane vesicles. To decipher the molecular mechanism behind these nuclear dynamics, we are conducting a whole-genome LC-specific RNAi screen for animals exhibiting defects in lamina dynamics. Surprisingly, we have identified genes involved in cell cycle regulation and cytokinesis, suggesting that cell division and cell death may be intimately linked. Because nuclear changes are prominent in a host of pathological settings in humans, we believe that our studies may identify factors relevant for these conditions as well.

194A **Sensing damage in the skin** Thomas SONNTAG[1], Shizue Omi[2], Claire Valotteau[3], Nathalie Pujol[21]CIML, Aix Marseille Univ, INSERM, CNRS, CIML, Turing Centre for Living Systems, Marseille, France, [2]CIML, Aix Marseille Univ, INSERM, CNRS, Turing Centre for Living Systems, [3]LAI, Aix Marseille Univ, INSERM, CNRS, Turing Centre for Living Systems

The skin is a physical barrier that provides protection from the outside world. Any breach of its integrity allows potential pathogens to enter. Animals therefore have evolved mechanisms to repair tissue and induce sophisticated protective immune responses. In common with all invertebrates, *C. elegan*s has only an innate immune system, but further, unlike insects, lacks motile immune cells to help repair barrier epithelia. On the other hand, its epidermis is surrounded by a stiff apical extracellular matrix (aECM), which has features in common with the cell walls of plants and yeast. The aECM exerts tension on the underlying tissues and counters hydrostatic pressure. In plants, cells constantly monitor the mechanical integrity of their cell wall during growth prompting the question of whether *C. elegans* does likewise for its aECM. Mechanical coupling between the aECM and the epidermis is known to be essential for embryonic elongation. In the adult, we have found that a decrease in skin stiffness results in the induction of an immune response in the epidermis*. We now intend to explore how mechanical inputs control tissue repair. We are probing the biophysical properties of the skin with Atomic force microscopy and nanoablation, and found, as predicted, stress anisotropy in the cuticle. In parallel, we have run a pilot suppressor screen for mutants that block the constitutive expression of the innate response reporter *nlp-29*p::GFP in the epidermis in furrow-less *dpy-7* mutants. One of the suppressors encodes the protein F09F9.2. It shares a C-Ter cysteine-rich domain with DPY-6, as well as 4 other secreted proteins, F01G10.9, F13B9.2, Y34B4A.10 and F33D4.6, but no other sequence similarity to other proteins. A transcriptional reporter shows that F09F9.2 is expressed in the epidermis and in a pair of neurons and their support cells. GFP-tagged F09F9.2 is secreted in the cuticle furrows. Abrogation of F09F9.2 in a mutant or through RNAi inactivation suppresses the constitutive expression of *nlp-29*p::GFP observed in all six furrow-less Dpy mutants, namely *dpy-2, dpy-3, dpy-7, dpy-8, dpy-9* & *dpy-10*, without affecting their Dpy phenotype, nor the absence of furrows. Several phenotypes have been ascribed to furrow-less mutants, including decreased stiffness, meisosome fragmentation, abnormal cortical cytoskeleton and altered permeability. Further epistasis analyses are needed to explain the role of F09F9.2 in regulating cuticle damage sensing.

*Aggad *et al.* eLife, 10.7554/eLife.75906, in press

195A **Actomyosin interconnectivity in body wall muscles** Ana Marta Silva, Daniel S. Osório, Fung Y Chan, Inês Lima, Rita Marques, Reto Gassmann, Ana X CarvalhoIBMC, Instituto de Biologia Molecular e Celular, and i3S, Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal.

A variety of actin crosslinkers, proteins that bridge actin filaments, coexist in the same tissue but the collective contribution of actin crosslinkers to the mechanical properties of distinct cellular actomyosin networks is poorly studied in vivo. The muscle sarcomere is an actomyosin network that is highly dependent on interconnectivity and several proteins with actin crosslinking activity have been described to be mutated in human muscle disease. α-actinin is thought to be the major actin crosslinker in muscle, crosslinking not only actin filaments but also titin, a giant protein that interconnects the center of the sarcomere with its edges. C. elegans body wall muscles are responsible for animal movement and sarcomeres have similar structure and protein composition to their vertebrate counterparts. Interestingly, a null mutant of the sole C. elegans α-actinin (ATN-1) is able to develop functional body wall muscles that, although presenting some anomalies, only mildly impair animal motility. This suggests that other proteins work alongside α-actinin to ensure sarcomere interconnectivity. In this study, we took advantage of the atn-1 null mutant and used it as a sensitized background to perform a 3D-motility-based RNAi screen of 19 actin binding proteins that express in muscle and crosslink actin in vitro. We found that depletion of 6 candidate proteins aggravated the burrowing ability of atn-1 null animals. Analysis of sarcomeric structure by live imaging of animals expressing fluorescent reporters

of muscle confirmed that atn-1 null muscles depleted of one of the hits, vinculin, presented much stronger sarcomere defects than non-depleted muscles. Defects included decreased number of sarcomeres, disorganized sarcomeric structure, and actin aggregation, and became more severe as animals aged. We are currently investigating how the α-actinin-titin-myosin axis for force propagation is affected by depleting vinculin in atn-1 mutants that do not bind actin or titin. With this study we expect to improve our mechanistic understanding of actomyosin networks in vivo and to significantly expand our knowledge on synergistic effects between F-actin crosslinkers, which have so far been mostly studied individually and in vitro. This will contribute to a better understanding of sarcomere interconnectivity that is key for muscle function and impacts both genetic muscle diseases and muscle loss due to aging or chronic diseases.

196A **Routine immunostaining, fluorescence in situ hybridization, and expansion microscopy in *C. elegans* enabled by cuticle removal using spatially-restricted enzymatic digestion** Chi Zhang[1,2], Yangning Lu[1,2], Madison A. Sneve[1,2], Ruihan Zhang[1,2], Edward S. Boyden[1,21]McGovern Institute, Massachusetts Institute of Technology, [2]Howard Hughes Medical Institute, Massachusetts Institute of Technology

Immunostaining and fluorescence in situ hybridization (FISH) of *C. elegans* is challenged by the impermeability of its cuticle to protein and RNA labeling reagents. Here we report a new method that uses hydrogel-anchored proteases to fully permeabilize the cuticle, which enables robust staining of protein epitopes and RNAs throughout the entire intact animal across developmental stages while preserving tissue morphology.

For protein staining, both exogenously expressed and endogenous protein epitopes can be stained; we demonstrated successful staining of endogenous epitopes of the nucleolar protein DAO-5 and exogenously expressed fluorescent protein epitopes throughout worm bodies. Colocalization analysis confirmed that immunostaining signals were consistent with expected localization patterns of the respective proteins.

For RNA-FISH, we demonstrated the staining of *myo-3* mRNA and the colocalization of mRNA signals with MYO-3::GFP in myofilaments in muscle cells and the staining of *unc-25* RNA and the colocalization of mRNA signals with UNC-25::GFP in GABAergic neurons, throughout the entire intact animal.

Furthermore, we performed expansion microscopy on cuticle-permeabilized and immunostained *C. elegans*, which resulted in more uniform expansion of adult animals (<5% versus >30% distortion) with a higher expansion factor (~6- versus 2.8-fold) and reduced experimental time (6 versus 17 days) compared to the previous method (*eLife* **2020** doi: 10.7554/eLife.46249).

Overall, this cuticle permeabilization method transforms cuticle-enclosed *C. elegans* into soft tissues for improved and routine immunostaining, fluorescent in situ hybridization, and expansion microscopy, making it a valuable tool for future studies.

197A **An adapted MS2-MCP system for live imaging endogenous cytoplasmic mRNAs** Cristina Tocchini, Susan Elisabeth MangoBiozentrum

Subcellular mRNA localization is an evolutionarily widespread phenomenon involved in different aspects of post-transcriptional gene regulation and protein allocation. Although a growing number of localized transcripts has been uncovered, the dynamics and the mechanisms through which the localization gets achieved are largely unknown. Live imaging of RNA molecules constitutes an invaluable means to understand the dynamics through which transcripts can localize subcellularly, but live imaging in *Caenorhabditis elegans* has been difficult to achieve. Endogenous transcripts have been observed in nuclei, but endogenous mRNA has not been detected in the cytoplasm.

We have adapted live imaging methods to visualize mRNA in embryonic epithelial cells. We have tagged endogenous transcripts with MS2 hairpins that are bound by fluorescent MCP proteins, and detected both nascent transcripts in nuclei and mature RNAs in the cytoplasm. Analysis of diverse constructs have revealed rules to achieve functionally tagged mRNAs. Although our focus has been epithelial cells during morphogenesis, this method can likely be applied for other cell types and stages.

198A **Characterizing the subcellular distribution of SOD-1 in *C. elegans* cells and tissues** Lucia Sedlackova[1], Jeremy Vicencio[1], Daisuke Chihara[1], Nicholas Stroustrup[1,21]Centre for Genomic Regulation (CRG), [2]Universitat Pompeu Fabra (UPF)

Superoxide dismutase 1 (SOD1) is an antioxidant enzyme that is prone to misfolding and gain of function toxicity implicated in neurodegenerative diseases. SOD1 is ubiquitously expressed and within cells freely diffuses in the cytosol and the mitochondrial intermembrane space where it catalyzes detoxification of superoxide, a toxic radical species. Misfolding of SOD1, which can be triggered by mutation, endoplasmic reticulum stress and oxidation, precedes its aggregation and mislocalization to intracellular compartments[1]. The identification of SOD1 as a player in the pathology of neurodegenerative diseases led to studies focusing on its role within the nervous system, leaving other tissues largely understudied. To characterize SOD-1 subcellular localization in

adult nematodes, ageing and stress, we tagged *sod-1* with a wrmScarlet fluorophore at its endogenous locus. We find that the *C. elegans* SOD-1 protein is widely expressed in soma throughout the larval stages and adulthood. A diffuse cytosolic distribution was observed with two exceptions, the coelomocytes, in which SOD-1 localizes to vesicular compartments, likely endosomes, and in hypodermis, in which SOD-1 co-localizes with mitochondria. SOD1 localization to these organelles was previously only reported from models of SOD1 pathogenesis, as a result of aberrant binding to mitochondrial membrane proteins[2] or a system for aggregated protein removal[3]. To date, neither localization has been associated with normal functionality of the native SOD1 protein. In summary, we have generated a model to study cell-specific distribution patterns of SOD-1 in *C. elegans* and report that subcellular distribution of the enzyme varies across cell and tissue types in patterns previously only associated with disease. Further study in this model could lead to elucidation of mechanisms driving distinct forms of SOD-1 subcellular localization in cells susceptible to different sources of stress and the outcomes of SOD1 mislocalization in pathogenesis.

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199A **Deletion of *flwr-1* suppresses *rab-37* mutant hypersensitivity to inhibitors of cholinesterase** Kevin J Kruse[1,2], Erik J Jorgensen[1,21]School of Biological Sciences, University of Utah, [2]Howard Hughes Medical Institute

The small GTPase Rab26/Rab-26 recycles synaptic vesicles through the autophagy pathway in flies and mice. The C. elegans ortholog of Rab26/Rab-26, RAB-37b, is expressed primarily in neurons, though its function in worms is unknown. Overexpression of GFP:: RAB-37b in *C. elegans* neurons causes overgrowth and excessive branching of neuronal processes, with large GFP accumulations in cell bodies. A strain obtained from the Oklahoma *C. elegans* Gene Knockout Project (strain RB1433), in which a major portion of the *rab-37* locus (including exons 2-4) is deleted, causes delayed and reduced egg laying, resulting in a slowly growing population of animals. Deletion of *rab-37* also makes animals hypersensitive to inhibitors of cholinesterase (*hic)*. At 20° C median survival time for rab-37 deletion mutants was 60 minutes, compared to 90 minutes for wild type (N2). I performed an ENU screen to identify suppressors of the *rab-37* deletion. As *rab-37* mutant worm populations are slow growing, suppressors were identified from populations of animals that grew faster than plates of *rab-37* deletion animals. An early candidate is the tetraspan protein FLWR-1 (formerly known as F20D1.1), an ortholog of the conserved Flower/Fwe protein in humans, mice, and flies. Flower is thought to be a calcium channel that cycles between synaptic vesicles and the plasma membrane and is required for activity dependent bulk endocytosis. Deletion of *flwr-1* in *rab-37(-)* animals restores egg laying and rescues the *hic* phenotype.

200A **HDAC inhibition combats neurodevelopmental trafficking stressors** Caitlin Taylor, Kang ShenStanford University

Neurons are large, complex cells that face unique membrane trafficking challenges both during rapid developmental outgrowth and over the course of a long functional lifetime. We have previously shown that the recycling endosome protein RAB-10 and the unfolded protein response regulator IRE-1 are essential for dendritic development in the *C. elegans* sensory neuron PVD: loss of either *rab-10* or *ire-1* results in a dramatic reduction of the PVD dendritic arbor and defective trafficking of membrane proteins. The broad importance of membrane trafficking in neuronal health is underscored by many studies linking endolysosomal and secretory genes to both neurodevelopmental and neurodegenerative diseases, and we sought to uncover novel mutations that can protect developing neurons from membrane trafficking stressors. We performed a forward genetic modifier screen using the null allele of *rab-10* and isolated a loss-of-function allele of *sin-3* which rescued the dendritic defects of *rab-10. sin-3* functions in a histone deacetylase complex (HDAC), which suggests a neuroprotective role for HDAC inhibition during dendritic development. HDACs are major regulators of chromatin structure that repress transcription by deacetylating histones, and we hypothesize that HDAC inhibition results in increased transcriptional flexibility that may be beneficial to combat cellular stress. We found that perturbing a SIN-3/HDA-3 complex rescued morphological and trafficking defects caused by both *rab-10* and *ire-1,* suggesting that HDAC inhibition can compensate for multiple cellular stressors, including ER stress and endolysosomal dysfunction. Our subsequent genetic and cell biological analyses suggest that HDAC inhibition protects against different cellular stressors via two distinct mechanisms, by altering endolysosomal trafficking outcomes and improving the processing of misfolded proteins. We hypothesize that SIN-3/HDA-3 inhibition promotes alternative cell biological pathways that allow diseased neurons to compensate for multiple membrane trafficking defects. We propose that transcriptional regulation of these compensatory developmental stress responses can help combat neuronal dysfunction.

201A **Bilayer lipid metabolism and chromatin-based membrane remodeling seal holes in the nuclear envelope** Sarah R Barger[1], Han Yang[2], Shirin Bahmanyar[21]Molecular Cellular and Developmental Biology, Yale University, [2]Yale University

After open mitosis, holes in the nuclear envelope occupied by spindle microtubules must be sealed to establish nuclear compartmentalization. The inner nuclear membrane protein, LEM-2, recruits CHMP-7, which serves as an adaptor for ESCRT membrane remodeling machinery to execute sealing. The transient accumulation of LEM-2/CHMP-7 to nuclear envelope holes is conserved across multiple organisms; however, loss of CHMP-7 gives rise to subtle or no defects in nuclear permeability suggesting redundant mechanisms are responsible for sealing holes. Using the stereotypical divisions of early *C. elegans* embryos, we found that CNEP-1, the nuclear envelope-associated regulator of the phosphatidic acid phosphatase lipin, contributes to nuclear closure by controlling metabolic changes in bilayer ER/nuclear envelope lipids. Interestingly, loss of *cnep-1/*lipin results in intranuclear membranes that originate at sealing sites suggesting a role for lipid metabolism in remodeling membranes around chromatin. Using endogenous CRISPR-mediated mutagenesis, we show that the interaction between LEM-2 and the DNA crosslinking protein BAF prevents membranes from abnormally wrapping mitotic chromosomes during nuclear envelope sealing. Strikingly, when both LEM-2/BAF binding and *cnep-1/*lipin are absent, enwrapped mitotic chromosomes are persistent causing irregular nuclear shapes and enhanced nuclear leakiness. EM tomography revealed abnormal stacks of nuclear membranes and nuclear envelope ingressions in embryos lacking CNEP-1 and LEM-2/BAF binding. Importantly, nuclear formation defects resulting from disrupted lipid metabolism are specific to loss of the LEM-2/BAF interaction - deletion of *chmp-7* or the domain of LEM-2 that binds CHMP-7 results in comparatively subtle defects. Together, our data support a mechanism in which bilayer lipid metabolism and membrane remodeling mediated by chromatin-LEM-2 interactions prevents abnormal membrane wrapping of chromatin for proper sealing. We are currently investigating how lipid composition and fatty acid saturation of nuclear membranes cooperates with chromatin-based membrane remodeling for hole closure.

202A **A BORC-dependent molecular pathway for vesiculation of cell corpse phagolysosomes** Gholamreza Fazeli[1], Roni Levin-Konigsberg[2], Michael C Bassik[2], Christian Stigloher[1,1], Ann M. Wehman[31]University of Wuerzburg, [2]Stanford University School of Medicine, [3]University of Denver

Phagocytic clearance is important to provide cells with metabolites and regulate immune responses, but little is known about how phagolysosomes finally resolve their phagocytic cargo of cell corpses, cell debris, and pathogens. While studying the phagocytic clearance of non-apoptotic polar bodies in *C. elegans*, we previously discovered that phagolysosomes tubulate into small vesicles to facilitate corpse clearance within 1.5 hours. Here, we show that phagolysosome vesiculation depends on amino acid export by the solute transporter SLC-36.1 and the activation of TORC1. We demonstrate that downstream of TORC1, BLOC-1-related complex (BORC) is de-repressed by Ragulator through the BORC subunit BLOS-7. In addition, the BORC subunit SAM-4 is needed continuously to recruit the small GTPase ARL-8 to the phagolysosome for tubulation. We find that disrupting the regulated GTP-GDP cycle of ARL-8 reduces tubulation by kinesin-1, delays corpse clearance, and mislocalizes ARL-8 away from lysosomes. We also demonstrate that mammalian phagocytes use BORC to promote phagolysosomal degradation, confirming the conserved importance of TOR and BORC. Finally, we show that HOPS is required after tubulation for the rapid degradation of cargo in small phagolysosomal vesicles, suggesting that additional rounds of lysosome fusion occur. Thus, by observing single phagolysosomes over time, we identified the molecular pathway regulating phagolysosome vesiculation that promotes efficient resolution of phagocytosed cargos.

203A **DRP-1 mediates microvillar effacement induced by Enterohemorrhagic *Escherichia coli*** Cheng-Rung Huang[1], Ting-Chiao Chen[2], Chang-Shi Chen[11]Institute of Biochemistry and Molecular Biology, National Cheng Kung University, [2]National Cheng Kung University

Enterohemorrhagic *E. coli* (EHEC) induces the characteristic «attaching and effacing» (A/E) lesion that results from actin cytoskeletal remodeling. Compared with the mechanism of EHEC-induced microvillar attachment, which has been thoroughly investigated over the past several decades, the underlying mechanism of EHEC-induced effacement of intestinal microvilli remained largely unknown. Our previous study showed that a signaling pathway involving mitotic cyclin-dependent kinase 1 (CDK-1) and diaphanous-related formin 1 (CYK-1) confers EHEC-induced microvillar effacement in *Caenorhabditis elegans*. Here we revealed that Dynamin-related protein 1 (DRP-1), a key regulator of mitochondria fission and a substrate of CDK-1, is required for EHEC-induced microvillar effacement in *C. elegans*. Furthermore, we demonstrated that DRP-1 involves in the CDK-1-formin signaling pathway to regulate EHEC-induced actin (ACT-5) redistribution. Taken together, our results support the notion that the CDK-1-DRP-1-formin signaling axis is necessary for EHEC-induced microvillar effacement, and mitochondrial dynamics may play a role.

204A **Changes in nucleolar architecture and biological functions during *C. elegans* aging** Laeya Baldini, Steph WeberMcgill university

The nucleolus is the most prominent organelle in the nucleus of eukaryotic cells. It coordinates the biogenesis of ribosomes. Recent progress has revealed the nucleolus to be a multilayered condensate formed through phase separation. This description offers new and unexpected insights into the biophysical rules governing its compartmentalization. However, how the architec-

ture and biophysical properties of the nucleolus dictate its function, and whether these change throughout an organism's life cycle, remains poorly understood. Thus, our research focuses on studying changes in the architecture and activity of the nucleolus during *C. elegans* aging. We use confocal microscopy and quantitative image analysis to monitor changes in nucleolar architecture throughout adulthood. Preliminary results demonstrate that the nucleolus exhibits rearrangement from a core-shell architecture to inverted cap during aging, disrupting the ordered layers thought to be critical for coordination of ribosome biogenesis. Moreover, RNA fluorescence in situ hybridization shows that this change is associated with a decrease in pre-rRNA synthesis. The nucleolus may progressively adapt and play other critical functions to cope with the physiological challenges that arise during the aging process. One hallmark of aging is the loss of protein homeostasis. Since the nucleolus has been shown to prevent protein aggregation during heat shock, we hypothesize that the nucleolus acts as a storage compartment for nuclear protein aggregates to protect cells from proteotoxicity during aging. Our next steps will be to evaluate protein aggregation by immunofluorescence. In addition, we aim to characterize the different stages of nuclear protein aggregation during aging by quantitative imaging and to monitor its reversibility by FRAP. Ultimately, we aim to determine whether the nucleolus exerts a protective effect on organism by reducing protein aggregation toxicity. We will study the impact of nucleolar architecture changes on the worm's physiological state by measuring lifespan and functional declines.

205A **Identification of new apoptosis regulators in *C. elegans*** Nadin Memar[1,2], Afroza Aman[1,2], Ralf Schnabel[3], Anton Gartner[1,21]Institute for Basic Science - Center for Genomic Integrity, [2]UNIST, [3]Institut für Genetik, TU Braunschweig

Apoptosis, the major form of programmed cell death, is important for the development of multi-cellular organisms. Defects in its regulation and execution have been implicated in a wide variety of diseases such as autoimmune diseases or cancer. Genetic analyses in *C. elegans* led to the isolation of apoptosis genes, and the elucidation of a conserved core apoptosis pathway needed during somatic development, and in the germ cells, where apoptosis is also induced by DNA damaging agents, and meiotic recombination failure. Even though many details of the apoptotic pathway have been worked out, its regulation is still not fully understood. Previous genetic screens were based on the isolation of apoptosis mutants that are viable under laboratory conditions.

We take advantage of a collection of temperature sensitive (ts) embryonic lethal mutants, to identify new regulators of programmed cell death that were missed in previous genetic screens because they are not only important for programmed cell death but also essential for embryonic development. We performed a secondary screen of the ts mutant collection from the Schnabel laboratory using semi-automated 4D microscopy to generate recording of the entire embryonic development which can be subsequently used to dissect the cell death pattern in these mutants with the software SIMI°BioCell. Using this approach, we were able to isolate a new candidate, *ced-x(t3481ts)*. This mutant exhibit excessive apoptosis during embryogenesis and in the germline, a phenotype also observed in a mutant carrying a loss-of-function mutation in the anti-apoptotic *ced-9*/BCL2 gene. Backcrossed *ced-x(t3481ts)* complements a *ced-9* loss of function allele, and sequencing of the *ced-9* locus in the *ced-x(t3481ts)* mutant did not reveal any mutation. These results suggest that *ced-x(t3481ts)* is a second locus besides *ced-9* that protects somatic and germ cells from apoptotic death.

206A **Regulation of centriole number by the microtubule remodeling factor SSNA-1** Jason Pfister[1], Lorenzo Agostini[2], Naoko Mizuno[2], Kevin O'Connell[11]NIDDK, NIH, [2]NHLBI, NIH

The centrosome functions as the primary microtubule-organizing center (MTOC) of the cell and plays important roles in ciliogenesis and assembly of the mitotic spindle. It is composed of an orthogonally oriented pair of barrel-shaped structures known as centrioles surrounded by a dense proteinaceous matrix called the pericentriolar material (PCM). In mitotic cells, centrioles duplicate once per cell cycle in a process that is highly regulated to insure proper centriole number. Improper duplication, resulting in either too few or too many centrioles, disrupts spindle structure and cilia function. Indeed, abnormal centriole number has been linked to cancers, primary microcephaly, and a variety of ciliopathies. Work in *C. elegans* has identified a core group of conserved proteins essential for centriole biogenesis; among these is the master regulatory kinase ZYG-1, a homolog of Plk4. Here we report our molecular and genetic analysis of the *C. elegans* protein SSNA-1, whose human ortholog has been shown to function in neuronal branching and microtubule remodeling. *In vitro* studies demonstrate that, like its vertebrate counterparts, worm SSNA-1 forms high-order oligomers that are essential for its function and can bind and branch microtubules. Using CRISPR-mediated genome editing, we have deleted the *ssna-1* gene and find that this results in a significant decline of embryonic viability and the formation of multipolar spindles. While our analysis indicates that the loss of SSNA-1 results in centriole overduplication, we find that *ssna-1* and *zyg-1* genetically and physically interact in complex manner suggesting that SSNA-1 plays both positive and negative roles in centriole assembly. In particular, we find that loss of SSNA-1 alters centriolar composition such that centrioles have less ZYG-1 at a time when overduplication is occurring. Finally, we have localized SSNA-1 in the embryo and find that during the first cell cycle SSNA-1 is restricted to centrioles; however, during the subsequent divisions, SSNA-1 also localizes to novel satellite-like structures that surround the PCM.

207A **The nucleoporin NPP-14 is required for co-clustering of nuclear pores and P granules in the *C. elegans* germ-**

line Laura L Thomas, Geraldine L SeydouxMolecular Biology and Genetics, HHMI / Johns Hopkins University School of Medicine

P granules are germline-specific condensates that enrich small RNA pathway components, including Argonautes. In the pachytene region of the germline, P granules are perinuclear and overlay clusters of nuclear pores (Sheth *et al*, 2010, Development). This organization has been proposed to facilitate surveillance of nascent germline transcripts by the small RNA machinery in P granules.

We have identified the non-essential nucleoporin NPP-14, a conserved cytoplasmic filament component, as playing a key role in P granule/nuclear pore co-clustering. In *npp-14* mutants, nuclear pores appear more uniformly distributed across the nuclear envelope, resembling the distribution typically seen in somatic nuclei. P granule coverage of nuclei is uneven and no longer correlated with nuclear pores. In addition, P granules in *npp-14* mutants are often detected in the rachis (the shared cytoplasm in the pachytene region). Interestingly, we observed a similar phenotype in mutants deficient for MIP-1, a recently identified P granule protein (Cipriani *et al*, 2021, eLife; Price *et al*, 2021, eLife).

Our observations suggest that clustering of P granules and nuclear pores is interdependent and requires both nuclear pore and P granule components. We are currently using the *npp-14* mutant to investigate the function of P granule coverage of nuclear pore complexes in the germline.

208A **Cortical actomyosin flow driven polarization, what goes with the flow and why?** Zeno Messi[1], Rukshala Illukkumbura[1,2], Nisha Hirani[1], Nathan Goehring[11]Polarity and Patterning Networks Lab, The Francis Crick Institute, [2]Leica Microsystems

A key requirement for polarized cells is an ability to pattern the localization and activity of molecules along the polarity axis. This can be achieved through a variety of mechanisms including reaction-diffusion-like processes or localized protein production. In many cases, molecules are polarized by active transport. One such transport mechanism is cortical actomyosin flow (CAF) as seen during the polarization of the C. elegans zygote. CAF segregates a set of conserved membrane-associated proteins, the PAR proteins (PAR-3, PAR-6, PKC-3), into the nascent anterior, breaking symmetry, allowing a second set of complementary PAR proteins (PAR-1, PAR-2 and LGL-1) to associate with the posterior membrane. A key question is how segregation by CAF is rendered selective to allow differential segregation along the polarity axis. To address this question, we leverage advantages provided by C. elegans zygote, including genome-engineering tools, a well understood polarity network, reproducible polarization geometry, and ease of imaging. First, we focus on the context of PAR polarity. Recent data indicate that segregation of anterior PAR proteins is linked to oligomerization of the scaffold protein PAR-3. We therefore address what features render PAR-3 uniquely sensitive to segregation by CAF. We aim to distinguish between several models for segregation, including differential coupling to the actin cortex, differential membrane association, positive feedback, and the role of PAR-3 oligomerization. Second, we explore the landscape of molecules transported by CAF. We will assess diffusion, membrane association, and advection of diverse molecules in the C. elegans zygote, including both endogenous and synthetic molecules (engineered with tunable features, e.g. membrane affinity, oligomeric state, and/or actin binding). Thus, we will assess how kinetic behaviours define the efficiency of segregation, to what degree advection is a universal feature of membrane/cortex associated molecules in this system, and, extending this analysis to other cell types, whether these characteristics are context dependent. Our work will provide insight into how the PAR polarity network engages CAF to enable its polarization, and into the landscape and biophysical features of molecules transported by CAF. Thus, we will establish the system design principles that govern how CAF shapes molecular distributions in cells and tissues.

209A **PAM-1 interacts with the cell-cycle machinery to regulate meiosis and mitosis in *C. elegans*** Aidan Durkan[1], Sophie Lear[1], Sarah Bell[1], Annalise Koup[1], Molly Jud[2], Bruce Bowerman[2], Rebecca Lyczak[31]Biology, Ursinus College, [2]University of Oregon, [3]Biology, Ursinus Col

Puromycin-sensitive aminopeptidases are implicated in cell-cycle regulation in many species. PAM-1, the *C. elegans* homolog of this metalloprotease, is needed for timely meiotic exit and polarization of the anterior-posterior axis. PAM-1 interacts with WEE-1.3 in polarity establishment and oocyte maturation. WEE-1.3 is a kinase that negatively regulates the cyclin-dependent kinase, CDK-1, suggesting that PAM-1 may work with key cell-cycle machinery. Here we have examined the role of PAM-1 in meiosis and mitosis in both *pam-1* mutants and suppressed strains. In *pam-1* mutant embryos, delays are observed during meiosis II and mitosis, as well as defects in sister chromatid separation. We compared these phenotypes in strains carrying different *pam-1* suppressors, *spam-2* and *spam-3.* In both suppressed strains, there were improvements in both meiosis and mitosis, showing that these suppressors may regulate M phase. In our previous work, we determined that *spam-2* is a missense mutation in *wee-1.3* that does not seriously compromise its function. While we are still working to confirm the causative *spam-3* mutation, we have evidence that the strain contains a missense mutation in *cdc-25.1.* CDC-25.1 is the activating phosphatase that removes inhibitory phosphates added by WEE-1.3 to CDK-1. To further explore the interaction between PAM-1 and CDC-25.1, we inactivated c*dc-25.1* with RNAi in *pam-1* and *pam-1; spam-3* strains. We found that inactivation of *cdc-25.1* eliminated the suppres-

sion of *pam-1* normally observed in *pam-1; spam-3* strains, suggesting that CDC-25.1 is necessary for the suppression. We are currently exploring this interaction further by crossing *pam-1* and *pam-1; spam-3* strains with loss-of-function and gain-of-function *cdc-25.1* mutants. Our study suggests that PAM-1 may act to regulate the activity of CDK-1 during meiosis and mitosis.

210A **A primary microcephaly-associated *sas-6* mutation perturbs centriole duplication and ciliogenesis in *Caenorhabditis elegans*** Mary Bergwell[1], Amy Smith[2], Ellie Smith[3], Carter Dierlam[4], Ramon Duran[4], Erin Haastrup[4], Rebekah Napier-Jameson[5], Rory Seidel[4], William Potter[4], Adam Norris[5], Jyoti Iyer[41]Oklahoma Medical Research Foundation, [2]Pfizer Inc., [3]Technische Universität Kaiserslautern, [4]University of Tulsa, [5]Southern Methodist University

A previous study identified a missense mutation within the human *SASS6* gene to be associated with the incidence of primary microcephaly (MCPH) in a Pakistani family. However, the consequences of this mutation have never been investigated in the context of a multicellular eukaryotic animal model. Further, the effect of this mutation on ciliogenesis is yet to be determined. In our study, we used CRISPR/Cas9 genome editing to re-create the MCPH-associated *sas-6(L69T)* mutation in the nematode *Caenorhabditis elegans*. Our studies indicate that the *sas-6(L69T)* mutation affects ciliogenesis more severely than centriole duplication. Specifically, our studies reveal that centriole duplication is only mildly defective in *sas-6(L69T)* mutant worms. As a consequence, *C. elegans* carrying the *sas-6(L69T)* mutation do not exhibit any defects in brood size or embryonic viability. However, interestingly, introducing the *sas-6(L69T)* mutation into a temperature-sensitive *zyg-1(it25)* strain that is partially compromised for centriole duplication, causes an increased failure of centriole duplication, thereby leading to a robust increase in embryonic lethality. Ciliogenesis defects have been previously linked with the incidence of MCPH. Strikingly, analysis of ciliogenesis in worms carrying the MCPH-associated *sas-6(L69T)* mutation revealed that *C. elegans* phasmid cilia are significantly shorter in the *sas-6(L69T)* mutant worms, indicating that ciliogenesis is perturbed in the presence of this mutation. Further, *C. elegans* carrying the *sas-6(L69T)* mutation also exhibit dendritic defects and defects in chemotaxis towards butanone. Since *sas-6* is an essential gene, most mutations in *sas-6* yield sterility or embryonic lethality, thereby making it difficult to dissect the role of SAS-6 in cilia assembly and maintenance. Through our studies, we have identified a pathologically relevant *sas-6* allele that more selectively perturbs ciliogenesis. Therefore, in the future, this allele could serve as a novel tool to further probe the mechanism by which SAS-6 regulates ciliogenesis.

211A **A model for striated muscle disorders caused by variants of human laminA** Ellen Gregory[1], Shilpi Kalra[1], Trisha Brock[2], Gisele Bonne[3], GW Gant Luxton[1], Christopher Hopkins[2], Daniel Starr[11]MCB, UC Davis, [2]InVivo Biosystems, [3]Institut de Myologie

Striated muscle laminopathies caused by missense mutations in the nuclear lamin gene *LMNA* are characterized by cardiac dysfunction and often skeletal muscle defects. Attempts to predict which *LMNA* variants are pathogenic and to understand their physiological effects lags behind variant discovery. We used *C. elegans* as a model by introducing pathogenic human *LMNA* variants and variants of unknown significance at conserved residues within the *lmn-1* gene. Severe missense variants lead to reduced fertility and/or motility in *C. elegans*. Nuclear morphology defects were also evident in the hypodermal nuclei of many lamin variant strains, indicating a loss of nuclear envelope integrity. Phenotypic severity varied within the two classes of striated muscle missense mutations, but overall, variants associated with both skeletal and cardiac muscle defects in humans lead to more severe phenotypes in our model than variants predicted to disrupt cardiac function alone. We also identified a separation of function allele, *lmn-1(R204W)*, that exhibited normal viability and swimming behavior but had a severe nuclear migration defect. Thus, we established *C. elegans* models that can be used as avatars for human laminopathies and characterized human *LMNA* variants that lend new insight into lamin function during nuclear migration in normal development.

212A **APC/C^{FZR-1} is controlled at several levels in the germline** David Puerta[1,2], Sara Rivera-Martin[2], Jose Perez-Martin[2,31]Instituto de Biomedicina de Valencia (CSIC), [2]Instituto de Biologia Funcional y Genomica (CSIC), [3]Instituto de Biomedicina de Valencia CSIC

The Anaphase Promoting Complex/Cyclosome (APC/C) is a significant ubiquitin ligase in cell cycle regulation and differentiation. APC/C targets many proteins using its co-activators Cdc20 and Cdh1 (FZR-1 in *C. elegans*). We have discovered that in *C. elegans*, APC/C^{FZR-1} promotes the degradation of MES-4 once the germline stem cells (GSC) enter the meiotic program. Since MES-4 is part of histone methylation complexes required to maintain GSC stemness, we believe these proteins' APC/C^{FZR-1} mediated degradation is required for the correct mitosis/meiosis transition.

Interestingly, along the distal part of the germline, where MES-4 is enriched, the APC/C^{FZR-1} complex was also present, indicating that its activity should be controlled somehow. We have tried to describe which elements were involved in the apparent repression of the APC/C^{FZR-1} complex in the mitotic region of the gonad.

We have found that at least two mechanisms cooperate to down-regulate APC/C^{FZR-1} complex. On one side, the co-activator FZR-1 is kept low by the concourse of FBF proteins. In addition, the activity of the CDK complex down-regulates the activity of the APC/C^{FZR-1} complex.

In this communication, we will also describe how the Notch pathway, which maintains the stemness of GSC in *C. elegans*, impacts these two negative controls of the activity of APC/C^{FZR-1}.

213A **RG/RGG repeats in the *C. elegans* homologs of Nucleolin and GAR1 contribute to sub-nucleolar phase separation** Emily L Spaulding, Alexis M Feidler, Lio A Cook, Dustin L UpdikeMount Desert Island Biological Laboratory

The intrinsically disordered RG/RGG repeat domain is found in several nucleolar and P-granule proteins, but how it influences their phase separation into biomolecular condensates is unclear. We survey all RG/RGG repeats in *C. elegans* and uncover nucleolar and P-granule-specific RG/RGGmotifs. An uncharacterized protein, K07H8.10, contains the longest nucleolar-like RG/RGG domain in *C. elegans*. Domain and sequence similarity, as well as nucleolar localization, reveals K07H8.10 (NUCL-1) to be the homolog of Nucleolin, a protein conserved across animals, plants, and fungi, but previously thought to be absent in nematodes. Deleting the RG/RGG repeats within endogenous NUCL-1 and a second nucleolar protein, GARR-1 (GAR1), demonstrates these domains are dispensable for nucleolar accumulation. Instead, their RG/RGG repeats contribute to the phase separation of proteins into nucleolar subcompartments. Despite this common RG/RGG repeat function, only removal of the GARR-1 RG/RGG domain affects worm fertility and development, decoupling precise sub-nucleolar structure from nucleolar function.

214A **Genome-wide synthetic lethality RNAi screen identifies genes essential for survival in response to the loss of the *lem-3* or *slx-1* nucleases in *C. elegans*.** Stephane Rolland[1,2], Hajung Yun[3], Luthfiyyah Mutsnaini[1,2], Taekyung Kim[3], Anton Gartner[1,21]Institute for Basic Science - Center for Genomic Integrity, [2]UNIST, [3]Pusan National Univerisity

Faithful chromosome segregation requires that all DNA bridges that physically link chromatids are removed before the completion of cell division. These bridges can originate from persistent recombination intermediate, local under-replication or chromosome entanglement and are processed by the conserved BTR complex in S-phase, the SLX-1/MUS-81 nucleases in G2 and the GEN-1 nuclease in late anaphase. Despite these redundant mechanisms, some bridges commonly persist in late mitosis. We have previously identified in *C. elegans* the conserved midbody-tethered nuclease LEM-3/Ankle1 as part of a 'last chance' mechanism that processes these persistent DNA bridges just before the cell divides (Hong et al, 2018a (PMID: 29463814)). We have also previously shown that the combination of *lem-3* and *slx-1* mutations causes synthetic lethality (Hong et al, 2018b (PMID: 29879106)). This provided us with an inroad for a synthetic lethality genetic screen to identify genes that act with *lem-3* and/or *slx-1* to process persistent DNA bridges. Candidates might also include genes that affect meiotic recombination or genes whose depletion leads to cryptic defects in DNA repair and DNA metabolism, which are only visible in *lem-3* and/or *slx-1* mutant background. Using a COPAS Biosorter, we have so far screened 50% of the Vidal RNAi library (~6000 clones) and identified 164 primary candidates. Among the confirmed candidates, we found two genes coding for histone H4 variants and one gene coding for a histone H3 variant, which caused synthetic lethality specifically with the *lem-3* mutant.

We hypothesize that these synthetic defects are due to cryptic defects in DNA replication, fork stalling, or replication restart and that lethality in double mutants is due to a failure to process branched DNA molecules leading to excessive DNA bridge formation. We also expect to find genes directly required for LEM-3 regulation.

215A **Epithelia delimits glial apical polarity against mechanical shear to maintain glia-neuron - architecture** Cecilia G Martin[1], James S Bent[2], Aakanksha Singhvi[31]Medical Scientist Training Program, University of Washington, [2]Basic Sciences, Fred Hutchinson Cancer Center, [3]Basic Sciences Division, Fred Hutchinson Cancer Center

For an organ to maintain proper architecture and function, its different component cell-types must coordinate their cell-shapes with each other through life. While cell-intrinsic developmental mechanisms driving homotypic cell-cell coordination are known, how heterotypic cells collectively regulate cell-shape is less-clear. We report that, in a sense-organ, epithelial cells delimit and maintain polarity domains of contacting glia, and thereby, associated neuron shapes throughout life. We uncover a molecular pathway wherein epithelia-to-glia signaling dictates sense-organ architecture through animal life, and report striking heterogeneity in these interactions.

Briefly, Hsp co-chaperone UNC-23/BAG2 keeps epithelial apical domains from deforming with animal movement. These defects appear in adult animals and are progressive with age. Epithelial apical domains stretch aberrantly and progressively in adult unc-23 mutant animals, which in an FGFR-dependent manner, dislocates glial apical cytoskeleton proteins SMA-1/βH-Spectrin and actin. This alters glial apical polarity and cell shape, and concomitantly, associated neuron-ending shape. Notably, epithelial UNC-23 acts temporally at a developmental critical period to maintain glia-neuron shape in adults, and spatially within a defined anatomical zone to only affect head glia. Lastly, intervention in either epithelia, glia or neuron ameliorate or phenocopy unc-23 neural defects.

Epi/endothelia resist mechanical stress and contact glia-neuron units across central/peripheral nervous systems and species, and all components of the identified molecular pathway are conserved and disease-relevant. Thus, we posit that analogous epithelia-glia mechanobiological coupling may broadly regulate glia-neuron shapes through animal life.

216A **Intentionally left blank**

217A **Coordinating the Seam Cytoskeleton & Matrix: How the Worm Forms Alae** Trevor Barker, Meera SundaramGenetics, University of Pennsylvania

All exposed epithelial surfaces are lined by a lipid and glycoprotein-rich apical extracellular matrix (aECM). Some aECMs form hardened acellular structures like *Drosophila* denticles, *C. elegans* alae, and fish scales, but the mechanisms by which these acellular structures are shaped remain poorly understood.

Alae are ridges found in the lateral cuticle of L1s, dauers, and adult *C. elegans*. Our lab, in conjunction with Alison Frand (UCLA) and Ken Nguyen and David Hall (Albert Einstein College of Medicine), recently found that actin within the L4 seam cytoskeleton is used to pattern the overlying "pre-cuticle" matrix into regions of adhesion vs. delamination (Katz, Barker et al 2022). These differing regions are what lead to the peaks vs. valleys of the forming adult alae, respectively. The seam cytoskeleton includes longitudinal actin filament bundles (AFBs), and the seam aECM shows complimentary patterns. We hypothesize that an unknown set of proteins control the assembly of these AFBs, anchor them to the apical plasma membrane, and connect them to the matrix to cause delamination.

I recently discovered roles for VAB-10 (or spectraplakin) and the spectrin family proteins SPC-1, SMA-1, & UNC-70 in alae shaping. All of these proteins have domains that bind actin, that bind to the apical plasma membrane, and/or that connect to various other cytoskeletal factors. VAB-10A & VAB-10B appear immediately between the medial seam AFBs, and spectrins show several complementary patterns to medial and junctional AFBs. Furthermore, mutants & RNAi experiments for spectrins and VAB-10 have revealed alae defects. I am currently testing epistatic relationships to better understand the hierarchy among the involved proteins. This will elucidate their potential roles in organizing seam AFBs and transmitting information to the matrix to ultimately shape the alae.

218A **"Mirror-image symmetry" in centrosome migration during serial cell divisions in *C. elegans* mid-stage embryo** Takefumi Negishi, Hitoshi SawaNational Institute of Genetics

Proper orientation of cell division axis is critical for development and stem cell maintenance. To achieve this, control of centrosome (or spindle pole) dynamics and establishment of cellular polarity are essential. In typical cell divisions, centrosomes migrate symmetrically after duplication to become spindle poles. In some stem cells including *Drosophila* male germ stem cells and neuroblasts, one of the duplicated centrosomes is anchored to the plasma membrane with migration of the other centrosome to the opposite side (asymmetric migration) resulting in oriented cell divisions. Such asymmetric behaviour of centrosomes has not been reported in *C. elegans*.

This study focuses on serial mitotic division along the anterior-posterior (A-P) axis in mid-stage *C. elegans* embryos. Intriguingly, we found that the centrosomes showed the asymmetric migration as in stem cell divisions. But unlike those divisions, the asymmetry was observed in the opposite orientation between sister cells. At the end of telophase in the previous division along the A-P axis, the centrosome reached the plasma membrane on the anterior and posterior sides of the anterior and posterior daughter cells, respectively, and then anchored to the plasma membrane. During the subsequent division cycle, one of the duplicated centrosomes stayed near the anchored location while the other migrated to the opposite direction, resulting in "mirror-image symmetry" in the migration pattern of the centrosome between the sister cells. Eventually, both sister cells form the spindle along the anterior-posterior axis. This mechanism is likely to be suitable for the regulation of the division axis during serial mitotic divisions in developing embryos.

Additionally, we found that double knockdown of DSH-2 and MIG-5 homologs of Dishevelled, a key component of the Wnt signaling pathway disrupted the division orientation. These proteins are localized to the posterior side of the cells. We are analyzing their roles in mirror-image symmetry of centrosome migration.

219A **Contractile ring mechanosensation and its RhoA-dependent tuning during early embryogenesis** Christina Hsu, Gaganpreet Sangha, Wayne Fan, Joey Zheng, Kenji SugiokaDepartment of Zoology, University of British Columbia

The contractile ring regulates the physical partitioning of dividing cells, but its closure is often asymmetric and oriented along the body axis in animal tissues. The asymmetric ring closure plays important roles in morphogenesis because its disruption leads to abnormal tissue integrity and reduced cytokinesis robustness (Maddox et al., 2007; Morais-de-Sa and Sunkel, 2013). Although pioneering studies have reported several regulators, such as ANI-1/anillin, and theories explaining asymmetric closure, the mechanochemical regulations of the contractile ring remain unclear. Here, we report that intracellular and intercellular mechanical stress regulate asymmetric ring closure, and that RhoA activity tunes contractile ring mechanosensitivity. Using high-resolution 4D imaging and quantitative analysis, we found that ring closure velocity and time lag of furrowing onsets be-

tween the leading and lagging cortex determine the peak ring eccentricity in the zygote P_0 cell. Next, we sought to understand the mechanism of time lag generation. Our analysis of cortical myosin movement showed that the slower, lagging cell cortex experiences circumferential-axis compression first, followed by the ring-directed cortical compression. At the leading cell cortex, however, ring-directed cortical flow and compression were observed immediately. Using the *act-2(or295ts)* mutant, which exhibits ectopic cortical contractility, we found that non-ring-directed cortical compression delays ring-directed cortical flow and locally suppresses the cortex ingression. The ring-directed cortical flow is the key factor because its artificial inhibition by adhesive beads also induces asymmetric ring closure in symmetrically dividing cells. Our genetic and quantitative analysis suggests that the previously reported positive feedback loop among ring-directed cortical flow, myosin enrichment, and ring constriction (Khaliulin et al., 2018) is the mechanosensitive engine driving asymmetric ring closure. The mechanosensitive engine is inhibited by ectopic RhoA activity, as in *ani-1(RNAi)*, and is rescued by the mutation of RhoA activator NOP-1. This rule applies to asymmetric ring closure in both the P_0 and the two-cell stage AB cell, which rely on intracellular and intercellular mechanical stress to suppress ring-directed cortical flow, respectively. This study demonstrates the novel mechanical regulation of the contractile ring and paves the way to dissect cytokinesis regulation in later embryogenesis.

220A **Extracellular vesicle budding and fertility are regulated by distinct levels of TAT-5 lipid flippase activity and conserved domains of the Dopey protein PAD-1** Lauren Pitts[1], Alexander Nguyen[1], Julia Frondoni[1], Katharina B Beer[2], Ann M Wehman[1,21]Biological Sciences, University of Denver, [2]Rudolf Virchow Center, University of Würzburg

The P4-ATPase TAT-5 acts as a lipid flippase to maintain phosphatidylethanolamine (PE) asymmetry in the plasma membrane and reduce extracellular vesicle (EV) release from the surface of *C. elegans* cells. TAT-5 is also required for embryonic development and both male and female fertility, but how different levels of flippase activity regulate these processes was unclear. An associated protein, PAD-1, shares essential roles in PE asymmetry, EV release, morphogenesis, and fertility with TAT-5. However, instead of being positioned to move lipids like the multi-pass transmembrane protein TAT-5, PAD-1 is a large cytosolic protein with poorly understood domains, making it unclear how PAD-1 could regulate PE asymmetry in the plasma membrane.

Previous studies on a mammalian P4-ATPase showed that mutations in the DGET motif in the Actuator domain can lead to a 3-fold (D to T) to complete (E to Q) loss in lipid transport (Coleman et al., PNAS 2012). Therefore, we generated similar mutations in the DGET motif of TAT-5. We discovered that *tat-5(E246Q)*mutants were sterile, while *tat-5(D244T)* mutants produced embryos that arrested during development. Using degron-based reporters, we found that EV release was increased in *tat-5(D244T)* mutant embryos and that phagocytosis was also disrupted. These data suggest that a low level of flippase activity can support fertility, while a higher level of flippase activity is required to inhibit EV release, allow phagocytosis, and carry out embryonic development.

PAD-1 has a conserved N-terminal Dopey domain and a C-terminal leucine zipper-like domain, whose mammalian homologs have been shown to bind to kinesin1 and lipid membranes (Mahajan et al., Nat Comm 2019). We discovered that both the conserved N-terminal EWAD motif and C-terminal leucine zippers are required for fertility and to inhibit EV release from the plasma membrane. We predict that these domains could interact with TAT-5 to regulate its flippase activity and plan to test this. As human TAT-5 and PAD-1 homologs are associated with developmental disorders, it is important to determine how these proteins regulate each other to function in diverse cellular processes.

This work was funded in part by DFG WE5719/2-1.

221A **Meisosomes, folded membrane platforms, link the epidermis to the apical extracellular matrix** Nathalie PujolCIML, Aix-Marseille Univ, CNRS, INSERM, Turing Centre for Living systems

Apical extracellular matrices (aECMs) form a physical barrier to the environment. In *C. elegans*, the epidermal aECM, the cuticle, is composed mainly of different types of collagen, associated in circumferential ridges separated by furrows. We have shown that changes in the structure of the aECM, specifically in mutants lacking furrows, can mimic physical injury and natural fungal infection by activating an immune response characterized by the induction of antimicrobial peptides in the epidermis[1,2]. In parallel, we have shown that tissue repair is coordinated with this innate response, and involves myosin-independent actin ring closure guided by the microtubule-tip protein EB1 in the epidermis[3].

To understand further the communication between the aECM and the epidermis, we have characterised epidermal membrane stacks that are found specifically in cuticle-lined epithelia in the worm, that we term 'meisosomes,' in reference to eisosomes in yeast. Ultrastructural analyses revealed that these stacks are composed of several parallel folds of the epidermal plasma membrane, alternately filled with cuticle. In the main epidermal syncytium (hyp7), meisosomes are only present in the lateral and dorso/ventral ridges, in a distinct and complementary pattern to hemidesmosomes, which are found above the 4 quadrants of bodywall muscles. In all six furrow-less Dpy mutants, namely *dpy-2, dpy-3, dpy-7, dpy-8, dpy-9* & *dpy-10*, we observed that meisosomes are fragmented and that the normal intimate connection between the epidermis and the cuticle is lost, specifically at

the lateral and dorso/ventral ridges, where there are no hemidesmosomes. We propose that just as hemidesmosomes connect the epidermis above the muscles to the cuticle, meisosomes connect the lateral epidermis to it. Moreover, furrow mutants present marked modifications of the cortical cytoskeleton before moulting and the biomechanical properties of their skin revealed by Atomic force microscopy, in addition to the constitutive damage response in the epidermis mentioned above. As meisosomes co-localise to macrodomains enriched in phosphatidylinositol (4,5) bisphosphate (revealed by PLC-1δ-PH probe), they could conceivably act, like eisosomes, as signalling platforms, to relay tensile information from the aECM to the underlying epidermis, as part of an integrated stress response to damage[4].

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222A **Regulated endoplasmic reticulum remodeling inhibits ectopic RNP condensates in oocytes** Jennifer A Schisa, Mohamed T Elaswad, Mingze Gao, Nicholas Trombley, Cora Zoet, Christya Haddad, Chloe Munderloh, Corrin Hays, Grace Thomas, Neeke Busette, Ashley D›Amour, Lauren RupeCentral Michigan University

Infertility is a significant issue among women due to poor oocyte quality; however, the regulation of oocyte quality remains poorly understood. Our long-term goal is to determine if the dysregulation of RNA binding protein (RBP) phase transitions contributes to infertility. We are currently focused on dissecting the regulatory pathways that modulate RBP phase transitions during oogenesis. We have identified Extracellular Signal-Regulated Kinase (ERK) as a key inhibitor of condensation of multiple RBPs in maturing oocytes. In meiotically arrested oocytes, activated ERK is not detected, and condensation of several RBPs including the KH-domain MEX-3 protein occurs. Similarly, depletion of *mpk-1(ERK)* in maturing oocytes leads to ectopic condensation of several RBPs. To identify the mechanism by which ERK inhibits condensation, we are testing if the phosphorylation of MEX-3 by ERK inhibits MEX-3 phase separation. In parallel, we are testing if ERK phosphorylates subunits of the CCT chaperonin, a second inhibitor of ectopic MEX-3 condensation which contains consensus ERK docking domains. Because obligate substrates of the CCT chaperonin include actin and tubulin, we asked if *cct* condensation phenotypes are mediated by either cytoskeleton system. To date, we have detected ectopic MEX-3 and CAR-1 granules after depletion of *act-4*, suggesting the role of CCT may be independent of folding the RBPs themselves. Actin regulates the endoplasmic reticulum in multiple developmental contexts; therefore, we next examined the effects of depleting *mpk-1, cct,* and *act-4* on remodeling of the ER in maturing oocytes. In all three cases, the ER became significantly remodeled, consistent with the possibility that ectopic condensation of RBPs occurs due to ER remodeling, a model supported by studies showing membrane surfaces can control condensate assembly in diverse processes. For example, the ER contacts Processing-bodies and stress granules, and ER shape can affect the biogenesis of P-bodies. We are now testing if ectopic MEX-3 condensates in oocytes are tethered to the ER, and will ask if the translational capacity of the ER drives condensation of RBPs in oocytes. Ultimately, we hope to unravel the complex regulation of RBP condensation during oogenesis and the effects of regulated RBP condensation on oocyte quality.

223A **Intestinal depletion of *cdc-25.2* increases germ cell apoptosis through mitochondrial oxidative stress** Mijin Lee, Hyemin Min, Yhong-Hee ShimDepartment of Bioscience and Biotechnology, Konkuk University

Cell division cycle 25 (CDC25) phosphatases control cell division during developmental processes by removing inhibitory phosphates from the cyclin-dependent kinase. In the previous study, we reported that expression of *cdc-25.2* is germline-enriched at the adult stage to promote oocyte maturation. However, *cdc-25.2* is also expressed in a variety of somatic tissues at the post-developmental stage, suggesting its non-canonical role in the soma. Here, we investigated a role of *cdc-25.2* in the soma at the adult stage and its association with germ cell development. We first analyzed the loss-of-function *cdc-25.2(g52)* mutant and found that the level of germ cell apoptosis (GCA) was increased CEP-1 and CED-13-dependently but not EGL-1. Interestingly, *cdc-25.2* RNAi in DCL569 (germline-specific RNAi) or in NL3511 (soma-biased RNAi) increased the level of GCA, suggesting that somatic CDC-25.2 non-autonomously regulates GCA. To confirm soma-to-germ line interaction in this process we performed intestine-specific *cdc-25.2* RNAi and examined GCA because the intestine is known to be a major signaling organ. Intestine-specific *cdc-25.2* RNAi indeed increased the level of GCA, induced intestinal leakage and mitochondrial oxidative stress, which were suppressed by an antioxidant, N-acetyl-L-cysteine (NAC) treatment, suggesting that mitochondrial oxidative stress causes intestinal leakage and GCA. Furthermore, the increased level of GCA was significantly suppressed by intestinal overexpression of *cdc-25.2* in *cdc-25.2(g52)* mutants. Therefore, these findings indicate that CDC-25.2 in the intestine controls intestinal integrity and GCA by modulating mitochondrial oxidative stress. In addition, the level of p-PMK-1 was significantly increased in the intestinal cells by intestine-specific *cdc-25.2* RNAi, which was also suppressed by NAC treatment. Our results suggest that mitochondrial

oxidative stress induced by the intestine-specific *cdc-25.2* RNAi activates PMK-1 in the intestine, and increases the level of GCA. Based on these findings, CDC-25.2 in the intestine non-autonomously regulates GCA by controlling mitochondrial oxidative stress through intestinal PMK-1 activity. This study was supported by NRF2021R1A2C1011658.

224A **Caffeine intake reduces reproductive capacity through germ cell proliferation, apoptosis and autophagy by regulating vitellogenesis in *C. elegans*** Juhae Kim, Hyemin Min, Mijin Lee, Yhong-Hee ShimDepartment of Bioscience and Biotechnology, Konkuk University

Caffeine (1,3,7-trimethylxanthine) is one of the most widely consumed bioactive molecule in the world. Studies have shown that caffeine intake has both beneficial and adverse effects on *C. elegans* development and aging. In our previous study, we have shown that maternal caffeine intake reduces fertility and oocyte quality by decreasing the level of vitellogenin in the intestine. In this study, we further evaluated effects of caffeine intake on reproduction. We found that caffeine-fed-hermaphrodites showed a reduced number of germ cells with decreased number of PH-3-postive cells in the distal region of gonad, and the increased number of PGL-1-absent germ cells with co-localized LGG-1 foci and CED-1::GFP-positive germ cells in the pachytene region of gonad. These results suggest that caffeine intake reduced germ cell proliferation (GCP) and increased the level of germ cell apoptosis (GCA). The increased level of GCA by caffeine intake was further elevated by UV treatment, indicating that caffeine-induced GCA was independent of DNA damage pathway. We also confirmed that caffeine-induced apoptosis was independent of *cep-1*, a regulatory gene for DNA damage-induced apoptosis, and mRNA levels of its downstream genes, *egl-1* and *ced-13* were not altered. Considering that caffeine intake reduces vitellogenesis, we performed RNAi depletion of *vitellogenin* gene 2 (*vit-2*), and found that GCA was significantly increased. These findings suggest that caffeine intake reduces reproductive capacity by decreasing GCP and increasing GCA and autophagic activity in the germ line through reduction in vitellogenesis in the intestine during germ cell development. This study was supported by NRF2021R1A2C1011658.

225A **Nuclear Vesicle Release During Neuronal Extrusion Events** Rebecca J Androwski, Barth D. Grant, Monica DriscollMolecular Biology and Biochemistry, Rutgers University

Accumulation and aggregation of cellular material is a hallmark of many diseases associated with aging, most notable of which is Alzheimer's disease. A growing body of work describes the effects of Alzheimer's disease on the neuronal nucleus and on the genome. Alzheimer's disease brains show an accumulation of nuclear localized proteins, elevated DNA fragmentation, and a higher prevalence of oxidative DNA damage compared to non-disease controls. Taken together, emerging data emphasize the nucleus as an area of critical attention for Alzheimer's disease research. Mechanisms of nuclear quality control, especially under stress or with age, however, remain poorly understood.

C. elegans provides a unique in vivo perspective on mechanisms of neuronal waste disposal. Previously the Driscoll Lab described a unique capacity of *C. elegans* adult neurons to remove deleterious cell contents by extruding a strikingly large (~5µm) vesicle, called an **exopher**. Recent work increasingly supports an exopher-like mechanism for removing damaged and aggregating material that may be conserved across *C. elegans*, rodent, and human models. Exopher-like structures have been observed in human brains, with elevated numbers in brains of Alzheimer's disease samples. Furthermore, work in *C. elegans* suggests that the exopher serves a neuroprotective role; such that neuronal function is better maintained as the animals age when they produced an early exopher.

Unexpectedly, the touch neuron nuclei produce vesicles that coincide with exopher production, first appearing on day one of adulthood. These nuclear vesicles (NVs) initially remain attached to the nucleus by a tunneling nanotube-like structure but are contained within the exopher compartment and are finally extruded from the neuron for eventual degradation as a sub-compartment in the exopher. The NV does not include the nucleolus or the nuclear pore reporter NPP-12, but is surrounded by the nuclear inner membrane protein EMR-1. DAPI staining confirms that 53% of NVs contain DNA and EM serial sections show NV cargo similar in appearance to the condensed heterochromatin of the nucleus. Taken together our data describe a novel degradative cargo and demonstrate a potential mechanism through which nuclear contents are regulated in postmitotic, long-lived neurons.

Ongoing NV projects explore how the nucleus maintains proteostasis, copes with age-related DNA damage, and responds to pathologically relevant nuclear aggregation models.

226A **NanoBRET in *C. elegans* – a novel technique to illuminate protein trafficking** Victoria E Groß[1], Miron Gershkovich[2], Anette Kaiser[2], Torsten Schöneberg[3], Simone Prömel[11]Institute of Cell Biology, Heinrich Heine University Düsseldorf, [2]Institute of Biochemistry, Leipzig University, [3]Rudolf Schönheimer Institute of Biochemistry, Leipzig University

The investigation of proteins, especially their interactions and trafficking, is of great interest for understanding general molecu-

lar processes and complex cellular functions. Excellent methods are established to study these aspects *in vitro*. However, they are mostly not applicable to *in vivo* settings, although these reflect the protein behavior in its natural environment. One of the available tools is bioluminescence resonance energy transfer (BRET), which is based on the close proximity of a luciferase that catalyzes a light-emitting reaction a fluorophore that is excited by the light. When both are fused to proteins and these interact, luciferase and fluorophore get into proximity and fluorescence can be detected. Here, we established NanoBRET in *Caenorhabditis elegans* to monitor protein-protein interactions and protein trafficking *in vivo* and in real-time. NanoBRET uses the small engineered Nanoluciferase, which is brighter than other luciferases.

First, we generated the basis for BRET in *C. elegans* by testing uptake and distribution of different luciferase substrates in the tissue for stable luminescence levels over a reasonable time span. For BRET establishment, we chose two different receptors as test proteins, NPR11 and LAT-1, and their known interaction partners, which are peptides. Both receptors have different expression patterns and functions in the nematode. They were endogenously expressed coupled to the Nanoluciferase and then investigated on the ability to generate a BRET signal with their fluorescent ligands. Our results show that BRET can be used to detect protein interactions, but the suitability strongly depends on the interaction of interest.

Furthermore, besides protein-protein interactions, BRET is also used for monitoring subcellular localization and trafficking of proteins. Therefore, we used CAAX::GFP, a well-characterized biomarker for labeling cell membranes to show a ligand-induced internalization of NPR-11, supposedly towards endosomes, and re-cycling back to the plasma membrane in real-time.

With the use of BRET in *C. elegans*, now a tool is available that allows for the study of distinct ligand-protein and protein-protein interactions as well as protein trafficking in an entire organism in real-time helping to understand protein behavior in their natural environment.

227A **Identifying substrates of aPKC in the worm early embryo to elaborate anterior PAR protein network interactions** Iolo Squires, Josana Rodriguez, Aaron Brooks, John PackerNewcastle University

Identifying substrates of aPKC in the early embryo to elaborate anterior PAR protein network interactions

All metazoan cells must create asymmetries in cellular components (such as protein, RNA, structures). This requires evolutionarily conserved groups of proteins, known as PAR proteins, which form domains at the cell cortex to establish asymmetry by signalling downstream. The signalling roles is performed by kinase members of each group.

An important kinase of the PAR proteins is atypical protein kinase C (aPKC). Understanding aPKC's role in cellular asymmetry is essential for understanding biological processes including cell migration, tissue homeostasis, and during development. Additionally, dysregulation of aPKC has been associated with several diseases, including cancer, neurological disorders, and cardiovascular disease. Therefore increasing our knowledge of pathways this kinase regulates is vital. To understand aPKC further, we use the worm one-cell embryo as our model system.

The first division of the *C.elegans* embryo is asymmetric; this requires forming an anterior and posterior domain along the long axis of the embryo. The primary signaling component of the anterior domain is the kinase, aPKC. Our lab has found that an anterior active domain of aPKC requires its interaction with two complexes: one is responsible for aPKC's localization (the localization complex) and the other to activate aPKC (the activation complex). Intriguingly, aPKC's kinase activity is required for these interactions. Due to the kinase dependency of these interactions, we hypothesize that they require a substrate or interactor of aPKC, which we will identify.

We employed a comprehensive phosphoproteomic comparison between aPKC-inhibited and control embryos to determine aPKC's substrates. This approach revealed 159 genes that contained potential aPKC phosphosites. Through bioinformatic scoring of these genes according to: their GO term, aPKC motif match and fold change in phosphorylation, the 159 genes were narrowed down to 60 genes. To confirm these 60 shortlisted candidates have roles in the one-cell embryo, an RNAi screen was undertaken which identified 22 with early embryo phenotypes. Phosphomimetic and non-phosphorylatable mutants for these genes have been generated using CRISPR-Cas9. We will analyze these mutants for alterations to aPKC's interaction with the localization and activation complexes.

I will present the analyses of phosphomimetic and non-phosphorylatable mutants from these putative aPKC targets. This should identify mechanisms responsible for the dynamic exchange between the localization and activation complexes, which is key for the polarization of the embryo. Also, I will reveal aPKC substrates governing other aspects of polarity and embryonic development, providing a rich resource for future study.

228B **The nuclear cargo adaptor KASH protein UNC-83 regulates the choice of dynein vs. kinesin-1 motor activity to move nuclei in opposite directions during development** Selin Gümüşderelioğlu[1], Kyoko Chiba[2], Ellen Gregory[1], Natalie Sahabandu[1],

Shinsuke Niwa[2], Richard McKenney[1], GW Gant Luxton[1], Daniel A Starr[11]UC Davis, [2]Frontier Research Institute for Interdisciplinary Sciences

Nuclear movements driven by dynein and kinesin are important for many developmental events. Like most cargo, nuclei are simultaneously bound to both dynein and kinesin, resulting in their bi-directional movement along a microtubule with a net directionality towards one end. How nuclei bias their direction is poorly understood. We address this problem by studying bidirectional nuclear movement during *C. elegans* hypodermal development. In this model, the outer nuclear membrane KASH protein UNC-83 serves as a cargo adaptor to recruit both dynein and kinesin-1 to nuclei. While dynein is the major force producer for larval P-cell nuclear migration, kinesin-1 drives nuclear migration toward plus ends of microtubules in embryonic hypodermal precursors. It is unknown how UNC-83 regulates the choice of motors at different times in development. We hypothesize that the net directionality of nuclear movement in *C. elegans* is determined by the developmentally regulated expression of alternative isoforms of UNC-83. Mutations affecting the long UNC-83a/b isoform disrupts dynein-dependent movements during larval P-cell nuclear migration, while disrupting all isoforms affects both nuclear migrations. We found that residues 58-233 specific to the long UNC-83a/b isoform were necessary for dynein-dependent nuclear migration. Moreover, we found that a line expressing the long isoform under control of the short isoform's promoter disrupted nuclear migration in embryonic hypodermal precursors, indicating that the UNC-83a/b-specific domain was sufficient to disrupt kinesin-1 mediated nuclear migration. We then tested if the short isoform UNC-83c directly activates *C. elegans* kinesin-1 motor activity on microtubules using single molecule TIRF microscopy assays. The presence of UNC-83c increased kinesin-1 binding to microtubules and stimulated processive movements. In contrast, UNC-83a/b did neither. These results support our current model where UNC-83c directly activates kinesin-1 to move nuclei in embryonic hypodermal precursors, while UNC-83a/b counteracts UNC-83c's ability to activate kinesin-1, causing dynein to become the dominant motor during larval P-cell nuclear migration. This work is an important step towards understanding how nuclei coordinate bidirectional microtubule-dependent transport during development.

229B **Nuclei migrate through constricted spaces during hypodermal P-cell development using multiple mechanisms** Jamie Ho[1], Leslie Guerrero[1], Ellen Gregory[1], Linda Ma[1], Diana Libuda[2], GW Gant Luxton[1], Daniel A Starr[11]UC Davis, [2]Univ Oregon

Nuclear deformability is the limiting step for cell migration through narrow constrictions such as those in immune cell intravasation and cancer metastasis. The mechanisms that facilitate nuclear movement through narrow spaces are unclear. We established a model in the hypodermis of L1 C. elegans where P-cell nuclei migrate through a narrow constriction as a normal part of development. We previously showed that, linker of the nucleoskeleton and cytoskeleton (LINC) complexes recruit dynein, to the nuclear envelope to move nuclei toward the minus ends of microtubules. We hypothesized that new players could be identified that work parallel to the LINC complex through genetic screens. Here we report the identification of new proteins that function in P-cell nuclear migration. We identified five proteins that are thought to be involved in the regulation of actin networks. CGEF-1, a predicted CDC-42 guanine nucleotide exchange factor (GEF), and FLN-2, a divergent filamin, were both identified as enhancers of the nuclear migration defect in LINC complex mutants. Furthermore, knockdown of CDC-42, ARX-3 of the ARP2/3 complex, or non-muscle myosin-2 NMY-2 using the auxin-inducible degradation system leads to a P-cell nuclear migration defect, indicating that actin networks are necessary for nuclear migration. In addition to actin-based pathways, we identified a heterochromatin-mediated pathway. CEC-4 is an inner nuclear membrane protein that tethers H3K9 methylated chromatin to the inner nuclear envelope and is therefore predicted to affect the ability of the nucleus to deform. Mutations in *cec-4* or in methyltransferases *met-2* and *set-25* synergize with mutations in the CDC-42/CGEF-1 pathway and LINC mutants. In our model, three mechanisms work together to ensure P-cell nuclei can migrate through a constricted space: 1) LINC complexes recruit dynein to the nuclear envelope to move nuclei toward minus ends of microtubules; 2) CDC-42-regulated actin networks help deform nuclei; and 3) heterochromatin at the periphery of the nucleus provides structural support for migrating nuclei. This work reveals novel mechanistic insights into the fundamental process of confined cell migration during development with implications for human diseases, including cancer.

230B **Microtubule glutamylation: required for cold tolerance and male mating, dispensable for centrosome function** Jessica Lee[1], Bhumi Shah[1], Daniel Chawla[1], Nina Peel[21]TCNJ, [2]Biology, TCNJ

Glutamylation, the covalent attachment of glutamic acid to tubulin in the polymerized microtubule, is enriched on long-lived microtubules. It is thought to contribute to centriole stability, cilia motility and axon function. Glutamylation of the microtubules is catalyzed by a family of tubulin tyrosine ligase like (TTLL) enzymes. To investigate the function of tubulin glutamylation we have generated a *ttll-4(tm3310)*; *ttll-11(tm4059); ttll-15(tm3871) ttll-5(tm3360) ttll-9(tm3889)* 'quint' mutant that lacks all five *C. elegans* glutamylating enzymes. Glutamylation is undetectable in this quint mutant and the adult males show mating defects, likely due to impaired cilia function. Hermaphrodites do not, however, present a Dyf phenotype suggesting that amphid and phasmid cilia remain intact in the absence of glutamylation. Thus worm cilia show differential requirements for glutamylation. Although in other organisms glutamylation is enriched on centriolar microtubules, we cannot detect centriolar glutamylation in the worm. Moreover the localization of centriole markers ZYG-1, SPD-2 and SAS-4 is normal in the quint mutant and the centro-

some organizes a fully functional spindle. Our data therefore suggest that in *C. elegans* microtubule glutamylation is not essential for centriole stability or centrosome function. Interestingly, we find that the quint mutant shows enhanced colchicine and cold-sensitivity, suggesting that microtubule stability in inclement conditions is altered by the loss of glutamylation.

231B **LINC complexes at the nuclear envelope regulate organelle positioning by modulating cytoplasmic macromolecular crowding** Hongyan Hao[1], Xiangyi Ding[1], Patrick Alinaya[1], Shilpi Kalra[1], Liam Holt[2], GW Gant Luxton[1], Daniel A Starr[11]UC Davis, [2]New York University School of Medicine

Macromolecular crowding influences biochemical reaction rates and the biophysical properties of intracellular environments. Crowding is modulated in individual cultured cells by regulating ribosome concentrations. However, the mechanisms that regulate molecular crowding in multicellular *in vivo* contexts remain largely unexplored. We hypothesized that the linker of nucleoskeleton and cytoskeleton (LINC) complex is required to maintain cytoplasmic macromolecular crowding *in vivo*. LINC complexes consist of the KASH protein ANC-1 at the outer nuclear membrane and the SUN protein UNC-84 in the inner nuclear membrane. Deletions in the large spectrin-like domains of ANC-1 cause significant organelle positioning defects, where organelles appear completely unanchored and move freely throughout the cytoplasm. As *anc-1* mutant animals bend, hypodermal nuclei, ER, lipid droplets, and mitochondria "slosh" around the cytoplasm. To explore the biophysical properties of *in vivo* tissues, we used a novel tool, genetically encoded multimeric nanoparticles (GEMs), to perform tissue-specific single-particle tracking nanorheology experiments in the developing worm. GEMs are homomultimeric scaffolds fused to a green fluorescent protein that self-assemble into bright, stable particles 40 nm in diameter that can be imaged and tracked on a 10-20 ms time scale. We engineered *C. elegans* strains stably expressing cytoplasmic 40 nm GEMs under the control of tissue-specific promoters in the hypodermis, intestine, and neurons. The effective GEM diffusion coefficients were significantly increased in *anc-1*, *unc-84*, or *lmn-1* mutant intestinal and hypodermal cells, suggesting that LINC complexes are required to maintain the biophysical properties of the cytoplasm. Ribosomes are a major contributor to macromolecular crowding. Interestingly, we found that ANC-1 is required for the homogeneous distribution of ribosomes *in vivo*. Furthermore, two ribosome components, RPS-15 and RPS-18, were necessary for hypodermal nuclear positioning. Collectively, these results establish a role for the LINC complex and ribosomes in controlling the mesoscale biophysical properties of the cytoplasm *in vivo* across several different tissues. This is the most complete description of macromolecular crowding and its physiological implications in a multicellular context to date.

232B **Optimized dimerization of PAR-2 via its RING domain underlies cooperative membrane recruitment, plasma membrane selectivity, and feedback-driven cell polarization** Tom Bland[1], Nisha Hirani[1], David Briggs[1], Neil McDonald[1], David Zwicker[2], Nathan W Goehring[11]Francis Crick Institute, [2]Max Planck Institute for Dynamics and Self-Organization

The behavior of cell polarity networks are defined by the quantitative features of their constituent feedback circuits. These circuits must be appropriately tuned to create networks that not only robustly and stably polarize but remain responsive to dynamically changing cellular states and/or spatial cues that arise during development. Using the PAR polarity network as a model, we uncover a key role for the N-terminal RING domain of the polarity protein PAR-2 in mediating positive feedback via dimerization. Combining theory and experiment, we find that dimerization affinity is optimized to achieve dynamic, selective, and cooperative recruitment of PAR-2 to the plasma membrane during polarization. Consistent with this model both reducing or enhancing dimerization compromises polarization. Strikingly, increasing dimer affinity stabilizes membrane binding, which somewhat paradoxically leads to reduced PAR-2 loading onto the posterior plasma membrane during symmetry-breaking due to kinetic trapping of dimeric PAR-2 on internal membranes. Thus, our data reveal a key role for a dynamically oligomeric RING domain in optimizing interaction energies to support a robust and responsive cell polarity network.

233B **Characterization of a putative *C. elegans* ortholog of the ERAD protein, Valosin-containing Protein-interacting Membrane Protein (VIMP)** Caroline L Dahlberg[1], Tessa Marks[1], Anna Byquist[1], Lars Ellgaard[2], Alina Dhalla[31]Biology, Western Washington University, [2]University of Copenhagen, [3]Antelope Valley College

The ER-associated Degradation machinery consists of large protein complexes whose makeup can change depending on the stress level of the ER. The Valosin-containing Protein-interacting Membrane Protein (VIMP, SelenoS) is a member of the 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 used the ER stress reporter transgene, P*hsp-4*::GFP to determine if F26F4.9 is required for regulating *hsp-4* during an ER stress response. We find that a genetic deletion in F26F4.9 (*tm2433*) reduces the activation of P*hsp-4*::GFP that usually occurs when animals are treated with the ER stress inducing drug, Tunicamycin, or when animals are lacking other ERAD machinery. This is

particularly striking in animals with reduced HSP-4 (BiP) expression. RNAi against *hsp-4* dramatically increases the activity of GFP expressed under the *hsp-4* promoter. However, in RNAi-fed animals, the loss of F26F4.9 reduces the high P*hsp-4*::GFP expression by 50%. Our current work is addressing the mechanisms by which F26F4.9 may work within the ERAD machinery to regulate gene transcription as part of the unfolded protein response.

234B **Autophagy in meiotic fidelity and genome integrity in the *C. elegans* germline** Kaitlin Kosinski[1], Marilina Raices[2], Judith Yanowitz[3], Alicia Melendez[41]Biology, Queens College/City University of NY, [2]Department of OB/GYN/RS, University of PIttsburgh, [3]Department of Obstetrics, Gynecology and Reproductive Sciences, University of PIttsburgh, [4]Biology, Queens College, City University of NY

Autophagy is a conserved cellular recycling process crucial for homeostasis. In this multistep process, cellular material destined for degradation is enclosed in the autophagosome, a double membrane-bound organelle that fuses with the lysosome for degradation. Reproduction is an energetically expensive process for living organisms, and the decision for an organism to invest in its offspring or survival thus requires the communication between the conditions in the environment and the germ line. Autophagy provides a potential mechanism for signaling to the germline that environmental conditions require diverting resources for individual survival rather than reproduction. Although typically upregulated in response to cellular stress conditions, basal levels of autophagy can also function as a quality control mechanism that serves to maintain cellular homeostasis in response to developmental and environmental cues. The role of autophagy in cellular homeostasis is well documented, but its function in the maintenance of genomic stability in the germline remains unclear. We recently described roles for BEC-1, the *C. elegans* ortholog of mammalian BEC-1/BECN1, and several other autophagy genes in germline stem cell homeostasis (Ames et al., 2017). Specifically, we found that BEC-1*,* as well as ATG-18 (in mammals, WIPI1/2), ATG-16.2 (ATG16L) and ATG-7 (ATG7), are required for the late larval expansion of germline stem cell progenitors during development. We have also reported a role for BEC-1 in the DNA damage response (DDR) to UV, where it exhibits crosstalk with CEP-1, the *C. elegans* ortholog of p53 (Hoffman et al., 2014). CEP-1 is well known for its roles in promoting genomic integrity through the DDR, including cell cycle control, apoptosis, and DNA repair. CEP-1 also acts in meiosis, in the double-strand break formation and suppressing nonhomologous end-joining (NHEJ), to ensure the high fidelity repair of double-strand breaks (DSBs) by the error-free homologous recombination (HR) pathway (Mateo et al., 2016). We are now investigating the mechanism(s) by which BEC-1 and autophagy genes promote DNA damage repair in germline development. Our current work seeks to elucidate which tissues require autophagy to regulate meiosis and germline proliferation and at which step of meiosis autophagy genes are required to regulate the DNA damage response during germline development.

235B **Identifying new proteins that facilitate the incorporation of autophagosomes to phagosomes that contain apoptotic cells in C. elegans** Zheng Zhou[1], Omar Pena-Ramos[2], Lucia Chiao[21]Baylor Col Med, [2]Baylor College of Med

Autophagosomes are double-membrane intracellular vesicles that degrade protein aggregates, intracellular organelles, and other cellular components. During the development of the nematode Caenorhabditis elegans, many somatic and germ cells undergo apoptosis. These cells are engulfed and degraded by their neighboring cells. We discovered a novel role of autophagosomes in facilitating the degradation of apoptotic cells using a real-time imaging technique. Specifically, the double-membrane autophagosomes in engulfing cells are recruited to the surfaces of phagosomes containing apoptotic cells and subsequently fuse to phagosomes, allowing the inner vesicle to enter the phagosomal lumen. Blocking the incorporation of autophagosomes to phagosomes delays degradation. As our autophagosome-phagosome incorporation assay is capable of identifying the distinct autophagosome-phagosome recruitment and fusion defects, we performed a candidate gene screen for mutants defective in either the recruitment of autophagosomes on phagosomal surfaces or the fusion of autophagosomes to phagosomes. We have identified multiple RAB GTPases that play differential roles in the recruitment and fusion of autophagosomes to phagosomes. We have also identified other proteins with novel roles in the autophagosome-phagosome incorporation. This work will reveal the unique molecular mechanisms that drive the incorporation of double-membrane vesicles to single-membrane organelles and further elucidate how apoptotic cells are efficiently cleared from animal bodies.

236B **WormAtlas: New Chapters, New Data, New Worms** Nathan E Schroeder[1], Laura A Herndon[2], Cathy A Wolkow[2], Zeynep F Altun[2], David H Hall[21]University of Illinois at Urbana-Champaign, [2]Albert Einstein College of Medicine

The well-characterized anatomy of *C. elegans* has propelled its use as a model organism. Initially characterized through extensive light and electron microscopy (EM) observations, these data continue to provide insight into *C. elegans* biology. The Center for *C. elegans* Anatomy is a hub for the presentation and interpretation of anatomical data through the WormAtlas and WormImage websites. WormAtlas comprises multiple resources designed to assist users in understanding the structure of *C. elegans* and, ultimately, in interpreting their own anatomical data. During the past year, WormAtlas and WormImage logged over 264,000 pageviews from 42,737 users. Users came from 172 countries, with the U.S. having the largest user base, followed by China and India. The WormAtlas handbook chapters include tissue-specific descriptions of *C. elegans* anatomy. Originally, focused on the

adult hermaphrodite and male, we have expanded our handbooks on dauer anatomy and structural changes that occur during aging. During the past year, new chapters were written on muscle and the reproductive system in aging *C. elegans*. We have also begun extending our coverage of anatomy beyond *C. elegans* by recruiting members of the nematode research community to contribute chapters on *Pristionchus pacificus* and the human parasitic nematode *Strongyloides stercoralis.* In addition to our collection of physical EM archives of *C. elegans,* mostly now available in digital form on WormImage*,* we have begun retrieving archival EM data sets of other nematode species. These additional data sets include approximately 50,000 negatives and prints that are being digitized and annotated for future inclusion on WormImage. We have recently partnered with the NIH-funded BossDB image repository to host datasets used for the "Mind of the Worm" (White et al., 1986). To ensure the long-term viability of WormAtlas, the Hall and Schroeder labs are now working together to develop new tools and features. Together, we are co-hosting a workshop at this meeting on advances in EM techniques. Over the next year much of the physical archives will be transferred to the University of Illinois where they will remain accessible to the research community.

237B **Array Tomography facilitates ultrastructural data acquisition and analysis of *C. elegans* samples** Irina KolotuevEM facility, FBM, Université De Lausanne

Electron Microscopy (EM) is a powerful research tool extensively used in discoveries in *C. elegans* since the beginning of the field. Methods such as classical transmission EM, scanning EM-based volume methods, correlative light and electron microscopy, and immunoelectron labeling provide such invaluable information. Despite technological advancements, the potential of EM to address questions related to the *C. elegans* ultrastructure is not fully exploited. The reason is frequently that EM procedures are perceived as exceptionally complicated and time-consuming tasks requiring lengthy training. Given that many modern methods in biology are similarly complex and require specialized training, this view on its own is no longer valid.

Array Tomography (AT) is a relatively recent addition to the EM toolkit, offering a versatile technique that uses scanning EM to obtain transmission EM-like images. Most EM sample preparation methods are compatible with AT. Sample sections are transferred on a glass slide or a piece of silicon wafer, and observed in a scanning EM. Navigating the microscope chamber and screening the samples to localize the region of interest is more straightforward than the same procedure in the transmission EM. As the acquisition of serial sections and the subsequent alignment of the information in the 3D volume is easy to perform manually or semi-automatically, the importance of sectioning orientation is no longer crucial. In addition, the same sections can be analyzed using different immunolabeling procedures coupled to the ultrastructure data, making the correlation of information very accessible. AT combines the potential of different EM techniques to address any question that requires x-y resolution up to ~2 nm, making it a valuable tool for cell and developmental biology studies.

Many fields extensively use AT, but *C. elegans* is particularly suited for producing rapid results. In addition, the samples can be re-imaged after the first acquisition, as many times as necessary, using different acquisition conditions. I will present the image acquisition and image analysis strategies I employed in several recent studies. They include different embryonic and larval stages and various organs and body systems. The results can be analyzed with or without image segmentation while providing statistically significant sampling numbers. Volume datasets can be subsequently used by the entire *C. elegans* community on its own or as a reference for other studies.

238B **Ubiquitin modification in regulating endosome maturation** Mei Ding, Yue MiaoInstitute of Genetics and Developmental Biology, Chinese Academy of Sciences

The endosomal system constitutes a network of progressively maturing vesicles which are required for the degradation of membrane proteins and ionic channels. In the classical endocytic pathway, delivery of the endocytosed cargoes to lysosomes involves an endosome maturation process, which is mainly controlled by endosome-specific Rab GTPases and phosphoinositides. The maturation process from early endosomes to late endosomes is accompanied by an important switch in organelle identity marker, from Rab5 to Rab7. A key player involved in Rab5/Rab7 conversion is the SAND-1/Mon1-CcZ1 complex. This complex interacts with Rab5, PI(3)P, and Rab5 GEF Rabex5, causing dissociation of Rabex5 from the membrane, which probably terminates the positive feedback loop of Rab5 activation and then promotes the recruitment and activation of Rab7 on endosomes. Here, we utilized both *C. elegans* and mammalian cells and identified an ubiquitination related enzyme that reduces the level of GTP-bound active Rab5 and enhances the endocytic recruitment of SAND-1 to promote endosome maturation. Together, our data reveal a critical and direct role of ubiquitin modification in the endocytic trafficking process and have clear implications for the therapeutic treatment of related human diseases.

239B **ATFS-1 dependent mitochondrial UPR is required for viability of Caenorhabditis elegans lacking mitochondrial thioredoxin and glutathione reductases** Marina Valenzuela-Villatoro[1], David Guerrero-Gómez[1], Eva Gómez-Orte[2], Juan Cabello[2], Antonio Miranda-Vizuete[31]Instituto de Biomedicina de Sevilla (IBIS), [2]Centro para la Investigación Biomédica de la Rioja (CIBIR), [3]Redox Homeostasis Laboratory, Instituto de Biomedicina de Sevilla (IBIS)

Maintenance of redox homeostasis is essential for survival and, for this purpose, all eukaryotic organisms possess two major thioredoxin and glutathione/glutaredoxin redox systems, one located in the cytoplasm and the other in the mitochondrial matrix. While the cytosolic and mitochondrial thioredoxin reductases are encoded by different genes, the two isoforms of glutathione reductase are encoded by the same gene that has two different translation start codons. We have previously shown that the two cytoplasmic reductases TRXR-1 and GSR-1b have redundant functions in *C. elegans* molting, allowing the reduction of disulfide bonds within the worm cuticle to be shed (PMID: 21199936).

To explore whether mitochondrial glutathione reductase GSR-1a and mitochondrial thioredoxin reductase TRXR-2 also have redundant functions in *C. elegans*, our group has generated and characterized a double mutant *gsr-1a; trxr-2*. In contrast to the respective *gsr-1a* and *trxr-2* single mutants that have no overt phenotype, the double mutant *gsr-1a; trxr-2* has smaller size, a pale appearance and displays gonad migration defects. Consistently, *gsr-1a; trxr-2* mutants have a longer egg-lay period, produce less oocytes and their sperm has reduced fertilization capacity.

An RNAseq analysis on the *gsr-1a; trxr-2* double mutant versus wild type and single *gsr-1a* and *trxr-2* single mutants identifies a strong induction of a high number of genes related to a general defensive response, including genes involved in the immune response against pathogen infection or xenobiotics detoxification, as well as a large number of genes related to sperm function. Many of the genes induced in the double *gsr-1a; trxr-2* mutant are regulated by ATFS-1, the main transcription factor regulating mitochondrial UPR (UPR^{mito}) and, in consonance, *gsr-1a; trxr-2* animals strongly induce the *hsp-6* transcriptional reporter, the readout for UPR^{mito}. Notably, a triple mutant *gsr-1a; trxr-2; atfs-1* is lethal, demonstrating that the induction of the UPR^{mito} is essential for *gsr-1a; trxr-2* animals viability. In contrast, other survival pathways like UPR^{ER}, mitochondrial to cytosolic stress response (MCSR) or cytosolic heat shock response (CHSR) are only mildly disturbed in *gsr-1a; trxr-2* double mutants.

We are currently evaluating other phenotypes associated to mitochondrial dysfunction and dysregulation of redox homeostasis in *gsr-1a; trxr-2* double mutants.

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240B **Determining caspase functional conservation *in vivo*** Melissa Fee, Ginger Clark, Piya Ghose, Clay ClarkBiology, University of Texas at Arlington

Caspases (cysteine-aspartic acid proteases) are an ancient group of aspartate-directed cysteine proteases. Caspases have evolved from a common ancestor into two distinct subfamilies - initiator caspases and effector caspases. We sought to establish *C. elegans* as an *in vivo* system for examining evolutionary changes in caspase activity and regulation. To this end we overexpressed human caspase 3 in *C. elegans* touch neurons. Previous studies have shown that overexpressing *C. elegans* CED-3/caspase causes death of ALM neurons. We find a similar trend with the overexpression of human caspase 3. However, in addition, we find morphological defects of both the cell body and neurites. Future studies will include time-lapse studies, looking at a caspase activity reporter, as well as testing ancestral caspases using our paradigm.

241B **Investigating the role of Rab GTPase RAB-28 in extracellular vesicle biogenesis and glial uptake** Malek Elsayyid, Jessica E TanisBiological Sciences, University of Delaware

Secreted extracellular vesicles (EVs) are membrane-enclosed structures that transfer bioactive proteins, RNAs, and metabolites to recipient cells. Released from most, if not all cell types, EVs play critical roles in physiological processes and pathological conditions. In *C. elegans*, EVs are shed from primary cilia, specialized microtubule-based organelles that protrude from the dendritic tips of sensory neurons, and are environmentally released through a cuticle pore. EVs can also bud from the periciliary membrane of the ciliary base and be phagocytosed by surrounding glia[1]. Loss of the G-protein RAB-28 results in EV buildup within the extracellular space between the cilia and surrounding glia, suggesting its function as an EV shedding regulator[2]. Release of EVs containing the TRPP ion channel PKD-2 from the cilium distal tip is unaffected in the *rab-28* mutant. However, we have shown that male ciliated sensory neurons shed multiple different EV subpopulations, and that EVs containing the CLHM-1 ion channel are non-distal tip derived[3]. RAB-28 may discriminately regulate biogenesis of specific EV subpopulations. Using Total Internal Reflection Fluorescence (TIRF) microscopy and Imaris spot detection, I found that the number of CLHM-1-containing EVs released into the environment increased in *rab-28* (*gk1040*) mutants. Thus, I hypothesize that the CLHM-1 EV subpopulation may accumulate within the extracellular space in *rab-28* mutants and could therefore be phagocytosed by the glia. I used spinning-disk confocal microscopy to visualize tdTomato-tagged CLHM-1 expressed as a single copy transgene and observed colocalization of neuron-derived CLHM-1::tdTomato with p*miR-228*::GFP-labeled glial cells of the male tail sensilla. Currently, I am determining the impact of the *rab-28* mutation on CLHM-1 uptake by the glial cells. Characterizing mechanisms of EV biogenesis and the consequential effects on target cells is imperative to understanding this mode of intercellular signaling.

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242B **Determining the function of chromosomal nuclear peripheral localization in nuclear envelope rupture repair and cell cycle regulation** Ling Jiang[1], Karen Wing Yee Yuen[2], Yu chung Tse[31]school of biological science, The University of Hong Kong, [2]The University of Hong Kong, [3]Southern University of Science and Technology

The nuclear envelope (NE) protects genetic materials against cytoplasmic enzymes and regulates the transportation between the nucleus and cytosol in eukaryotes. The nuclear pore complexes (NPCs) can regulate chromosome architecture, genome integrity, gene transcription, and cell division. Depletion of specific conserved nucleoporins, including NPP-3/NUP205, NPP-7/NUP153, or NPP-13/NUP93, in *C. elegans* embryo cells, causes nuclear envelope rupture, and all condensed chromosomes to localize to the nuclear periphery, which also occurs in the anoxia and quiescence.

Upon nuclear envelope rupture by NPP-3 depletion, BAF-1 (Barrier-to-Autointegration factor-1) is first recruited to the rupture site which compacts chromatin and represses gene transcription. Then, LEM-2 and other repair proteins can accumulate at the rupture site for sealing. We have determined that the periphery localization of condensed chromosomes in NPP-3 depletion depends on BAF-1, centromere and kinetochore proteins, and spindle assembly checkpoint. We propose that such condensed chromosomes at the nuclear periphery is a protective mechanism to prevent DNA damage and degradation by cytosolic nuclease. In addition, NPP-3 depletion leads to an extended duration from nuclear envelope breakdown (NEBD) to anaphase onset. This delay is lost in the double depletion of NPP-3 and MDF-1/MAD-1, which illustrates that NPP-3 depletion can activate the spindle assembly checkpoint. Indeed, MDF-1 normally localizes to the nuclear envelope during prophase, but such localization is lost after NPP-3 depletion, but it remains unknown whether this is related to the activation of the spindle assembly checkpoint.

In this study, we will determine how cells respond to nuclear rupture, how they repair it, and which checkpoints are activated to protect the cells from damage. Furthermore, it also can provide a better understanding of the cellular pathology in diseases with nuclear envelope instability and frequent ruptures, such as in laminopathies and cancers.

243B **CCM-3 Regulates USO-1 to Promote Seamless Tube Extension** Ran Cheng[1,2], Bin Yu[1], Brent Derry[11]Development and Stem Cell Biology, The Hospital for Sick Children, [2]Molecular Genetics, University of Toronto

The excretory canal of *C. elegans* is an essential, single-celled tubular organ that forms an elongated “H” shape and runs the entire length of the worm. Canal structure consists of an outer basal membrane, a cytoplasmic region containing organelles and canicular vesicles, and an inner apical membrane that lines the lumen. The excretory canal is an excellent model for understanding fundamental mechanisms of tubulogenesis, which is relevant to the vasculature of vertebrate organisms. Previous work from our lab showed that the Cerebral Cavernous Malformations 3 (CCM-3) protein and its binding partner germinal center kinase 1 (GCK-1) are required for canal extension and maintaining its integrity through the regulation of membrane trafficking and the actin cytoskeleton, but how these processes are regulated remains obscure. To investigate this, we used a combinatorial approach that involved datamining existing protein-protein interaction datasets to create a CCM-3 “interactome”, then screening it using RNAi to look for canal truncations resembling those seen in *ccm-3* mutants. This strategy identified the vesicle transport factor *uso-1*, which is also genetically epistatic to *ccm-3*. Homozygous *uso-1* deletion worms have strong excretory canal truncations with cyst-like structures at the posterior tips. Specifically, *uso-1* ablated worms have dilated canal lumens and probable accumulations of canalicular vesicles in the cytoplasm, similar to those observed in homozygous *ccm-3* deletion worms. Taken together, this indicates that USO-1 plays an important role in maintaining excretory canal integrity downstream of CCM-3, and may be required for membrane trafficking that drives canal extension and maintenance of its integrity. A similar protein-protein interaction exists between human CCM3 and USO1, suggesting that this relationship is evolutionarily conserved. Human USO1 can be regulated by phosphorylation and has important roles in ER-Golgi trafficking and Golgi biogenesis that we intend to investigate. Based on these observations, we hypothesize that CCM-3 and GCK-1 regulate USO-1 to promote the ER/Golgi functions required for the membrane trafficking necessary for maintaining excretory canal integrity. Future work will focus on determining whether USO-1 is directly regulated by CCM-3 and GCK-1 and whether it is required for the integrity of other biological tubes in the worm.

244B **Intracellular transport of organelles in C. elegans** Anna GavrilovaFaculty of Biology, Medicine and Health, University of Manchester

Intracellular transport is the movement of cargoes such as proteins and organelles inside a cell. As neurons are one the largest

and most complex cells, the correct and efficient delivery of newly synthesized proteins from the cell body to distant locations in axons or dendrites is vital for neuronal function. Disturbances in axonal transport are linked to nervous system disorders and neurological diseases. Although much is known about how cellular vesicles are transported, predictive mathematical models for their movement are lacking. We have chosen to study neuronal transport in the nematode worms, Caenorhabditis elegans, which are convenient to maintain and image. We use a range of mutant strains of C. elegans: two kinesin light chain mutations (klc-1(ok2609), klc-2(km11)) and kinesin heavy chain mutation (unc-116(rh24sb79)). Additionally, we have generated new strains by performing crosses of the above strains with ida-1::gfp (BL5752) that has GFP-tagged dense core vesicles (DCVs) in the ALA neuron to obtain new datasets of organelle movement. We found that the transport properties of DCVs as well as the lifespan of our strains strongly depend on the mutations in kinesin light and heavy chains. Strikingly, the morphology of the ALA neuron changed after the cross of ida-1::gfp and unc-116 which needs further investigation. We transformed all our videos of the DCVs movement in different strains (more than 300 videos) into kymographs and tracked the particles using tracking software. This enables us to obtain a range of information such as velocities, rates of changing directions, rest states and more. Our experimental data shows that DCV movement in C. elegans is characterised by anomalous diffusion. Existing models of subdiffusion do not account for properties such as velocities and rates of direction switches, and hence cannot be applied to intracellular transport. Therefore, we want to build a persistent random walk model involving bidirectional movement with stochastic rest states that describes intracellular anomalous diffusion. This novel model will be used to analyse the existing data, and to predict the effects of minor changes in motor function, identified experimentally, on trafficking over long distances, such as in axons.

245B **The Spatial Organization of Sister Chromatids during Meiosis** Antonia Hamrick, Ofer RogBiological Sciences, University of Utah

Meiosis requires the parental chromosomes (homologs) exchange genetic information through the repair of DNA breaks, which subsequently allow their correct segregation. Each homolog is comprised of two genetically identical sister chromatids that could serve as the template. But, since sister-directed repair does not promote chromosome segregation, ensuring that the homolog is preferentially utilized is essential for successful meiosis. How the cell distinguishes the homolog from the sister, despite their near identity, remains a major mystery. When considering different models that account for the so-called 'homolog bias', a major unknown is the spatial organization of the sisters relative to one another. We have recently developed a technique to label individual sister chromatids using the nucleotide analog EdU, allowing us to describe the spatial organization of the sisters. Surprisingly, we found that in a mutant strain where the parental X chromosomes fail to pair (*him-8*), the sisters comprising the X chromosomes are differentiated from each other at the time of break repair. We will present our progress in measuring the extent of sister differentiation in the physiological, four-sister configuration, as well as potential regulators of sister differentiation, including the roles of meiotic progression, cohesins, and condensins. Understanding how the sisters are organized, and how to perturb this organization, will enable testing specific models that account for homolog bias and for the regulation of genetic exchanges. Furthermore, the mechanisms revealed by our work are likely to extend beyond meiosis, and impact the regulation of somatic DNA repair, gene expression, and mitotic cell division.

246B **EGFR Signaling Promotes Efferocytosis in *Caenorhabditis elegans*** Laura Filomena Comi[1], Silvan Spiri[2], Alex Hajnal[11]Department of Molecular Life Sciences, University of Zurich, [2]Department of Molecular and Cell Biology, University of California, Berkeley

The removal of cells undergoing apoptosis is a critical process in tissue homeostasis, development, and in the prevention of diseases. Defects in apoptotic cells recognition and degradation (efferocytosis) are the leading cause of autoimmune diseases and degenerative diseases. Therefore, it has been of major interest to study and further characterize genes involved in this process.

Pioneering work studying efferocytosis was done using the model organism *Caenorhabditis elegans*. In the *C. elegans* germline, physiological germ cell death is used to regulate the number of germ cells that can exit the pachytene stage of meiotic prophase I and enter oogenesis. After the CED-3 caspase has been activated, apoptotic germ cells expose "eat-me" signals. The dying germ cells are then recognized and engulfed by the surrounding somatic sheath cells, which eliminate them through efferocytosis.

Using an endogenously GFP-tagged LET-23 EGFR reporter, we observed an enrichment of LET-23::GFP, which is expressed in the sheath cells, around the apoptotic germ cell corpses. Furthermore, the EGFR ligand LIN-3 EGF is exposed at the cortex of apoptotic germ cells, and LIN-3 depletion from the germline resulted in the persistence of germ cell corpses. Likewise, inactivation of the *egfr* in the engulfing sheath cells using the FLP/FRT system caused an accumulation of germ cell corpses. Time-lapse and reporter analysis indicated that the increased number of corpses after sheath cell-specific inactivation of *egfr* is the result of a delay in corpse clearing due to a defect in early endosome fusion, rather than an increased rate in germ cell apoptosis.

Our findings suggest that EGF acts as an "eat-me" signal produced by the dying germ cells and recognized by the EGFR in the

engulfing sheath cells. We are further investigating the role of the EGFR and its downstream signaling pathway in the engulfment process and its interaction with other known "eat-me signals". Additionally, we are conducting experiments manipulating EGFR signaling in murine macrophages *in vitro* to investigate if we can translate our findings to mammalian systems.

Every day, billions of cells in our body undergo apoptosis. Efficient and accurate recognition and degradation of these cells is essential for our health. It is important to study and gain further understanding of efferocytosis to be able to design drugs treatments for these diseases and manipulate this process in a beneficial way.

247B **Substrate-specificity, and structure-function analysis of the conserved LEM-3/Ankle1 nuclease which ensures faithful chromosome segregation** Peter Geary[1,2], Junfang Song[3], Ye Hong[4], John Rouse[3], Stéphane G.M Rolland[1,2], Anton Gartner[1,21]Center for Genomic Integrity, Institute for Basic Science (IBS), [2]Ulsan National Institute of Science and Technology (UNIST), [3]MRC-PPU, University of Dundee, [4]Shandong Provincial Key Laboratory of Animal Cell and Developmental Biology, School of Life Sciences, Shandong University

Faithful chromosome segregation requires the removal of all DNA bridges that physically link chromatids before the completion of cell division. These DNA bridges can originate from persistent recombination intermediates, local under-replication, and chromosome entanglement. While several redundant safeguard mechanisms to process these DNA bridges exist throughout the cell cycle (from G2 to anaphase), some bridges often persist into late mitosis. We have previously identified, in *C. elegans,* the conserved midbody-tethered LEM-3/Ankle1 nuclease as part of a 'last chance' mechanism that eliminates these persistent DNA bridges just before the cell divides (Hong et al, 2018 (PMID: 29463814)); findings that were recently confirmed in human cells (Jiyang et al, 2023 (PMID: 36825683)).

We will present our recent data that provide insights into the substrate-specificity of LEM-3 as well as the molecular mechanisms underlying its recruitment to the midbody and its activity *in vivo* and *in vitro*. Specifically, we found that LEM-3 can cleave a wide range of branched DNA species *in vitro,* a result consistent with LEM-3 acting as a 'last chance catch-all' enzyme that processes DNA bridges caused by various perturbations of DNA metabolism. In addition, we found that the LEM (LAP2, Emerin, MAN1) and GIY-YIG nuclease domains of LEM-3 are required for its recruitment to the midbody, its activity *in vivo* and *in vitro* as well as its ability to bind to its DNA substrate. Sequence alignments and structure prediction by Alphafold also revealed the presence of three conserved, positively charged residues (K437, K441, and R456) on the surface of the LEM domain that might participate in DNA binding. We found that mutating one of these residues (*R456A)* reduced LEM-3 midbody localization and *in vivo* activity to a similar extent as deleting the entire LEM domain. In summary, we propose that the LEM domain of LEM-3 in addition to playing an important role in localizing this 'last chance catch-all' enzyme to the midbody, is also required for LEM-3 activity, likely by facilitating its DNA binding.

*Equal contributions to the work were made by Peter Geary and Dr. Junfang Song.

248B **The *hox* gene *lin-39* induces cell proliferation in somatic cells of *C. elegans*** Stefanie Engleitner[1], Simon Berger[2], Alex Hajnal[21]University of Zurich, [2]Department of Molecular Life Sciences, University of Zurich

lin-39 is a *hox* gene responsible for the differentiation and proliferation of the vulval precursor cells (VPCs) during larval development. In contrast to other metazoans, expression of *lin-39* and other *hox* genes is strongly down-regulated in most somatic cells that have terminally differentiated and exited the cell cycle. Previous results have shown that *lin-39* promotes cell cycle entry of the VPCs by directly activating Cyclin and Cdk gene expression (Roiz et al., 2016). Moreover, we recently found that over-expression of *lin-39* or *mab-5* is sufficient to cause the continuous proliferation of post-mitotic cells such as the uterine anchor cell (AC) or the sex myoblasts (SMs) (Heinze, manuscript in preparation). These findings have raised the possibility that prolonged expression of *lin-39* and other hox genes could maintain the proliferative potential of somatic cells until adulthood.

To investigate how constitutive *lin-39* expression in the AC promotes proliferation, we are conducting forward genetic enhancer screens for mutations causing increased AC proliferation. To this end, we are using newly developed microfluidic screening devices that allow the rapid imaging of large numbers of mutagenized worms at high resolution and the recovery of mutants of interest (Spiri et al., 2022). Through this approach, we will identify new factors acting with *lin-39* in a genetic network controlling AC proliferation. In parallel, we have performed RNA sequencing analysis of proliferating ACs and identified several candidate genes that may control AC proliferation. Furthermore, we will attempt to generate a C. elegans cell culture model by isolating GFP-labelled ACs and SMs from mutants that exhibit enhanced AC proliferation.

Our long-term goal is to understand the molecular mechanisms, by which hox genes control somatic cell proliferation during development and tissue regeneration.

249B **Heterodimerization of receptor-type guanylate cyclases is required for ciliary tip localization** Suzanne Rademakers[1],

Gert Jansen[21]Cell Biology, ErasmusMC Rotterdam, [2]cell biology, ErasmusMC Rotterdam

C. elegans senses salts in their environment using the ASE neurons. Detection of salts occurs in sensory organelles, called primary cilia. The receptor-type guanylate cyclase (rGC) GCY-22 is involved in the response to NaCl in the environment. We generated a full-length GFP knock-in in the *gcy-22* gene. GCY-22::GFP shows unique localization to the ciliary tip and periciliary membrane compartment (PCMC) of one ciliated neuron, ASER. Our goal is to understand the molecular mechanisms that regulate GCY-22 trafficking and unique ciliary tip localization. We set out to identify novel proteins that physically interact with GCY-22, by performing mass spectrometry after immunoprecipitation.

Five different ASER expressed rGCs are found to interact with GCY-22::GFP: GCY-3, GCY-4, GCY-5, GCY-13 and GCY-19. rGCs are thought to act as dimers and these findings suggest that GCY-22 might be a common subunit for heterodimeric complexes. To investigate the localization of each rGC and its role in the NaCl response, we generated GFP knock-ins and loss-of-function alleles. We found that GCY-3 and GCY-4 colocalized with GCY-22 at the PCMC, but not at the ciliary tip. GCY-13::GFP expression could not be detected. GCY-19::GFP colocalized with GCY-22::mCherry at the PCMC and ciliary tip and pull-down of GCY-19::GFP co-precipitated GCY-22. Loss-of-function of *gcy-19* resulted in lower levels of GCY-22::GFP at the ciliary tip and vice versa deletion of *gcy-22* resulted in lower GCY-19::GFP levels at the tip. Also GCY-5::GFP colocalized with GCY-22::mCherry and deletion of *gcy-22* resulted in reduced GCY-5::GFP levels at the tip. Pull-down of GCY-5::GFP co-precipitated GCY-22. Moreover, dendrite transport of GCY-22::GFP in *gcy-19; gcy-5* double mutants was severely affected. These results suggest that heterodimerization of rGCs is required for their correct trafficking and localization at the ciliary tip. Loss-of-function of *gcy-19* or *gcy-5* did not affect the animals' response to NaCl.

In addition, we identified DAF-25 in our GCY-22::GFP IP-MS experiments. DAF-25 is the ortholog of the mammalian ankyrin repeat and Mynd domain containing protein Ankmy2. DAF-25 has been reported previously to be important for rGC transport. Mutants lacking DAF-25 show no ciliary tip localization of GCY-22::GFP and do not respond to NaCl. To understand the mechanism of DAF-25 interaction with GCY-22, we performed DAF-25::GFP IP-MS. Candidate interacting proteins are currently being investigated.

250B **Structural and molecular determinants of dynamics in P granules of *C. elegans*** Stela Jelenic[1], Janos Bindics[1], Philipp Czermak[1], Balashankar R Pillai[1], Martine Ruer[2], Carsten Hoege[2], Alex S Holehouse[3], Shambaditya Saha[11]IMBA, Vienna, Austria, [2]MPI-CBG, [3]Washington University School of Medicine

The germline of sexually reproducing animals contains conserved non-membrane-bound compartments containing proteins and RNAs called 'germ granules'. Mutations that disrupt assembly of germ granules result in sterility. A germ granule in *C. elegans*, called 'P granule', has been shown to assemble via liquid-liquid phase separation (LLPS) of proteins and RNAs from the surrounding cytoplasm in adult gonads. Approximately 85% of proteins that concentrate in P granules are involved in different aspects of RNA metabolism, suggesting that the P granule phase could process and/or store RNA. To elucidate the underlying molecular mechanisms, it is important to understand the biophysical nature of the P granule phase. Specifically, the molecular and structural determinants that regulate diffusion rates within the P granule phase remain unclear.

We used an in vitro reconstitution-based approach to address this question and studied the LLPS behavior of the protein PGL-3, one of the most abundant proteins in P granules. We found that a folded domain of PGL-3 drives LLPS, and dynamics within phase-separated condensates correlates with alpha-helicity of the folded domain. Next, we investigated the effect on dynamics in condensates when other components of P granules are added to condensates of PGL-3. Our findings suggest that the complex composition of P granules favor fast dynamics within the phase.

251B **Enhanced Single RNA Imaging Reveals Dynamic Gene Expression in Live Animals** Erqing Gao[1,2], Jingxiu Xu[1], Suhong Xu[11]Zhejiang University-University of Edinburgh Institute, Zhejiang University-University of Edinburgh Institute, [2]College of Medicine and Veterinary Medicine, Deanery of Biomedical Sciences

Wounding induces immediate-early signals, including Ca^{2+}, ROS, and ATP, which play an important role in the maintenance of homeostasis. The expression levels of many genes could be upregulated after wounding. However, there is no suitable system to visualize this process since imaging endogenous mRNAs in live animals is technically challenging. Here we describe an MS2-based signal amplification with the Suntag system in *C. elegans* that enables live-cell RNA imaging of high temporal resolution and with 8xMS2 stem-loops, which overcomes the obstacle of inserting a 1,300 nt 24xMS2 into the genome for the imaging of endogenous mRNAs. Using this tool, we were able to image the activation of gene expression and the dynamics of endogenous mRNAs in the epidermis of live *C. elegans*.

252B **Role of LOTUS-domain proteins in liquid-like P granules** Balashankar R Pillai, Philipp Czermak, Shambaditya SahaIMBA

Germ cells ensure the continuance of life by giving rise to totipotent zygotes. A conserved feature of germ cells is the presence of perinuclear non-membrane-bound condensates of proteins and RNA, called 'Nuage'. Mutations in nuage proteins result in teratomas and cause infertility. Among conserved components of nuage are the ATP-dependent RNA helicase 'Vasa' protein, LOTUS domain proteins, Tudor domain proteins, and argonauts. The molecular mechanisms underlying the function of LOTUS-domain proteins in the germline remains poorly understood. In the nematode worm *C. elegans,* nuage is proposed to contain a collection of condensates – P granules, Z granules, SIMR foci and mutator foci. P granules contain two LOTUS-domain proteins MIP-1 and MIP-2, mutations of which reduces fecundity of animals. MIP-1 and MIP-2 each contain two LOTUS domains (hereafter called L1 and L2) in addition to two intrinsically disordered regions. Recent studies have identified a role of these intrinsically disordered regions in mediating association of P granules with nuclear pores. However, the role of the two LOTUS domains in MIP proteins remains unclear. Studies in Drosophila have shown that LOTUS domains can bind the Vasa protein and stimulate its RNA-dependent ATPase activity. Here, we show that in *C. elegans*, MIP-1 stimulates the RNA-dependent ATPase activity of the Vasa orthologue GLH-1. We also show that in vivo, L2 cannot complement for L1 function. We speculate that the LOTUS domains of MIP proteins have distinct roles in supporting the integrity and function of P granules.

253B **Identification of the molecular to functional consequences of human cytoplasmic actin variants** Theo Hecquet[1], Nadine Arbogast[1], Anaïs Goetz[1], Caroline Descamps[1], Nataliya Di Donato[2], Reymann Anne-Cecile[11]IGBMC, [2]TU Dresden, Institute for Clinical Genetics, University Hospital

Non-muscle actinopathies (NMA) are a set of ultra-rare human diseases caused by heterozygous single point mutations or gene deletion in cytoplasmic β or ɣ actin. Patients presenting NMA develop a wide range of symptoms with different severities, notably in terms of intellectual disability and facial or organ malformations. The genotype/phenotype correlations of NMAs are still unclear. Together with a team of collaborators working either with primary cell cultures or purified proteins, the Reymann team aims to understand the molecular to functional consequences of these cytoplasmic actin variants using the model organism *C. elegans.*

Nine human substitutions, chosen to span a large range of severity in patients, were successfully recapitulated in *C. elegans* actin-coding gene *act-2* using CRISPR/Cas9 mediated genome engineering. Interestingly, our preliminary results highlight that the general healthiness of mutant worms is correlated with patients' disease severity. Indeed, three variants corresponding to some of the most severe forms of NMAs caused homozygously non-viable strains. Using a variety of techniques, we assess the consequences of individual actin variants at different scales; from general worm fitness to embryo development up to *in vivo* molecular dynamics. Overall, we observed the presence of defects with different penetrance, such as increased embryonic lethality and reduced brood sizes. Accordingly, we observed mutation-specific gonad malformations and early embryogenesis defects regarding actin cortex structure and dynamics such as cell blebs or cytokinesis failure. We also observe variant-specific embryo arrest during, later, key events of development such as gastrulation or ventral enclosure. Nonetheless, we did not detect major changes in motility, touch response, or neuron positioning and identity (preliminary results) in surviving worms.

In humans most gain of function mutations lead to more severe symptoms than the actin gene deletion. A compensation mechanism is probably at work via the other non-affected genes. *C. elegans* offers us the possibility to investigate such possibility *in vivo.* We show that different actin knockout strains differ in terms of survival and fecundity questioning this hypothesis.

In conclusion, we show that *C. elegans* is a suitable model for the study of NMAs and we currently are integrating our *in vivo* results with those *in vitro* from our collaborators to propose some mechanism explaining the observed perturbations.

254B **Functional interactions between the apoptosis pathway and cell size are coordinated by the *ced-3* caspase – *ect-2* RhoGEF axis** Aditya Sethi[1,2], Hai Wei[2], Nikhil Mishra[2], Ioannis Segos[1,2], Barbara Conradt[1,21]Cell and Developmental Biology, University College London, [2]Faculty of Biology, LMU Munich

One of the emerging regulators of apoptotic fate is cell size. How cell size is controlled and how cell size can regulate apoptosis, however, remains unclear. We provide novel evidence that a key pro-apoptotic gene, *ced-3* caspase, interacts with the actomyosin regulator *ect-2* RhoGEF in neuroblasts that generate an apoptotic daughter cell to control the sizes of its daughter cells. Using a yeast two hybrid system and *in vitro* biochemical assays, we show that CED-3 caspase physically and directly interacts with ECT-2 RhoGEF. Epistasis analyses reveal that *ced-3* caspase most likely acts upstream of *ect-2* RhoGEF to promote asymmetric actomyosin enrichment in the neuroblast and thereby promotes the generation of a daughter cell that is small enough to undergo apoptosis. We also demonstrate that increasing the size of the apoptotic daughter cell by reducing *ect-2* RhoGEF function decreases its probability to undergo apoptosis. Conversely, we find that decreasing the size of the apoptotic daughter cell by hyperactivation of ECT-2 RhoGEF increases its probability to undergo apoptosis when the apoptotic pathway is partially compromised. However, decreasing the size of the apoptotic daughter cell does not increase its probability to die in animals where the apoptotic pathway is completely inactivated. These results suggest that a small size makes cells more prone

to die via apoptosis rather than another type of cell elimination. In summary, we have uncovered reciprocal and functional interactions between the apoptotic pathway and cell size: In neuroblasts, the apoptotic pathway acts upstream of *ect-2* RhoGEF to control the sizes of its daughter cells. Reciprocally, the small size of the apoptotic daughter cell promotes the activation/activity of the apoptotic pathway.

255B **Identification of new molecular players in the process of germline/soma distinction** Magali Nanchen, David Rodriguez-Crespo, Chantal WickyUniversity of Fribourg (Unifr)

In order to perpetuate themselves, sexual species need to produce functional haploid gametes. This process relies on proper distinction between germline and soma, which depend on the regulation of specific gene repertoires. Our lab characterized the zinc finger transcription factor LSL-1 as essential to produce functional gametes in *C. elegans*. It is expressed in germ cells from the birth of the P4 blastomere to the end of meiotic prophase I in the adult germline and it acts as a major transcriptional activator of germline genes involved in many aspects of germline development (Rodriguez-Crespo *et al*. 2022).

Further phenotypic analysis of *lsl-1* mutants revealed that germ cells are progressively reprogramed into neuronal cells as the worms grow older indicating that LSL-1 is essential for germ cell fate maintenance.

How LSL-1 is activating transcription at the molecular level is still unclear. Based on its early expression pattern in the P4 blastomere, we hypothesize that it may be one of the initial factors that activate germline gene transcription in primordial germ cells. LSL-1 could act by reading the epigenetic memory (H3K36 methyl marks) deposited in the parental germline.

To test this hypothesis, we are using two biochemical approaches. First, we immunoprecipitated LSL-1 using a GFP-trapping system to identify LSL-1 protein complex components by mass-spectrometry (CoIP-MS). Then, we established a proximity labeling approach based on TurboID to explore more broadly the chromatin context in which LSL-1 is binding.

Co-IP-MS revealed the protein BRA-2 as strong interactor of LSL-1. BRA-2 is homolog to the human ZMYND11 protein, predicted to be linked with histone methylation and transcription elongation regulation. *bra-2* mutants show phenotypic similarities with *lsl-1* mutants, i.e., sterility and high incidence of male. Additional candidates include three chromatin-associated proteins, XND-1, HIM-17 and MRG-1. All three proteins exhibit meiotic functions; however, XND-1 also functions as a germ cell determinant and MRG-1 is a barrier to germ cell fate reprogramming. Interestingly, MRG-1 is also a reader of histone mark H3K36 methylation, which is part of the germline epigenetic memory.

Finally, ongoing genetic interaction studies will allow us to determine whether LSL-1 and its interactors are functionally related. By these means, we intend to determine the molecular mechanisms by which LSL-1 regulate germline development and germ cell fate maintenance.

256B **Asymmetric distribution of actin-related proteins can precede known cell differentiation event in the early C. elegans embryo.** Grégoire Mathonnet, Roxane BENOIT, Delphine Sunher, Nadine Arbogast, Anne-Cécile ReymannIGBMC

During the early embryonic development of *C. elegans*, cell differentiation arises notably from cell polarization and asymmetric divisions. Specific segregation of maternally loaded molecules, such as polarity proteins or fate determinants, transcription factors or mRNAs, is occurring at each division and is key to drive early differentiation. Actin networks play a crucial role in these processes by tuning notably cortical properties at the cell periphery, thus affecting cell polarity and mechanics. However, how the actin related proteins are segregated between sister cells from the first asymmetric division onward remains poorly understood.

This study presents quantification of the spatio-temporal distribution of three essential actors of actin polymerisation: two nucleators, namely the CYK-1 formin and the Arp2/3 complex via ARX-2 as well as a capping protein CAP-1, from the zygote to the 4-cell stage. The use of CRISPR/Cas9 generated strains is allowing us to follow endogenous levels of proteins using quantitative image analysis. We also developed miniaturized *in vitro* actin polymerization assays using single-cell extracts to study actin nucleation capacities of each embryonic cell followed by fluorescent microscopy. This challenging technique is enabling us to access in more details the actin related content and the resulting dynamic for each cell. The combination of *in vivo* quantification and *in vitro* systems is enabling us to better assess the dynamic steady state of actin and the contribution of some actin binding proteins.

We found that temporal and spatial variation of master regulators of actin assembly do exist both in the cytoplasm and in the cortex, leading to unequal distributions of these proteins between sister cells. These data suggest that concentration gradients of freely diffusing actin binding proteins in the cytoplasm can exist; gradient which could impact the amount of actively recruited proteins regulating actin dynamics at the cortex. In particular, actin related content is lower in the posterior lineage. One of our

key results is that differences of content are revealed in the AB daughter cells, before the onset of cell signalling events from P2 that lead to ABp differentiation. Thus, asymmetric distribution of actin-related proteins can precede cell differentiation induced via intercellular signalling at this stage, opening a new scope for the role of actin in enabling cell identity acquisition in the early embryo.

257B **UNCovering the ciliary roles of C. elegans septins - UNC-59 and UNC-61** Emilia K Filipczak, Sofia Tsiropoulou, Oliver E BlacqueSchool of Biomolecular and Biomedical Science, University College Dublin

Primary cilia are microtubule based sensory and signalling organelles found on the surface of most eukaryotic cell types. The cilium's unique molecular composition is dependent on the Transition Zone (TZ) - a subdomain at the base of the organelle, which functions as a gated diffusion barrier to regulate the ciliary entry and exit of membrane and cytosolic proteins. Several TZ gating protein complexes are known such as the ciliopathy-associated MKS (at least 12 proteins) and NPHP (2 proteins) modules. Septins comprise a family of small G-proteins with roles in multiple cellular processes that includes membrane diffusion barrier regulation during cytokinesis. In vertebrates, septins regulate cilium length and signalling possibly via a role in controlling ciliary TZ gating. Here, we are investigating whether septins serve ciliary, including gating, roles in *C. elegans*. Although the two heterodimer-forming nematode septins, UNC-59 and UNC-61, are implicated in postembryonic cytokinesis, mitochondrial fission, and cell/axonal migration, ciliary roles have not been investigated. Employing knockout deletion alleles of *unc-59 (tm1928, tm1939)* and a nonsense allele of *unc-61 (e228)* we found that septin loss disrupts phasmid (but not amphid) cilia structure; also, phasmid cilia are misplaced due to abnormally short dendrites. Double mutant analysis suggests that septins do not functionally interact with MKS and NPHP module proteins and septin loss does not affect TZ protein localisation. However, assessment of ciliary gating using a ciliary excluding reporter shows a mild ciliary gating defect. In addition, localisation analysis shows weak or no expression of *unc-61* and *unc-59* in ciliated sensory neurons. Hence, it appears that the regulation of cilia by septins does not occur via an MKS/NPHP pathway and it may happen via cell non-autonomous mechanisms. Together, these data provide the first evidence of ciliary roles for nematode septins in a subset of ciliary structures.

258B **Deciphering the role of LGL-1 in *C.elegans* epithelial cells** Olga Jarosinska[1], Amalia Riga[1], Hala Fahs[2], Suma Gopinadhan[2], Fathima Refai[2], Anika van der Zant[1], Kristin Gunsalus[2], Mike Boxem[11]Utrecht University, [2]New York University Abu Dhabi

Cell polarity, driven by complex interactions between evolutionary conserved proteins, is essential for the proper cell and tissue functioning. Lethal giant larvae (Lgl) is one of the key regulators of cell polarity, and is also involved in asymmetric cell division, junction formation, and cell differentiation and migration. In mice and *Drosophila* loss of Lgl is lethal. However, the elimination of the *Caenorhabditis elegans* ortholog, LGL-1, has no effect on viability or development of the animals. The only known functions of LGL-1 are during the one cell stage of the embryo where it acts redundantly with PAR-2 to promote the asymmetrical segregation of the PAR proteins, and in the spermatheca where LGL-1 loss can suppress a *pkc-3* temperature sensitive mutation. To identify genes acting redundantly with *lgl-1*, we performed a whole-genome RNAi screen for synthetic phenotypes in an *lgl-1* deletion background. We found that the loss of PAC-1, a RhoGAP regulator of CDC-42, leads to embryonic lethality when combined with LGL-1 loss. The double loss of LGL-1 and PAC-1 causes the mislocalization of junctional proteins in the epidermis and leads to lethality during the embryonic elongation stage. Therefore, our results suggest a role for LGL-1 in the establishment and/or maintenance of junctions in the *C. elegans* epidermis.

259B **Investigating the role of apoptosis in maintaining progeny fitness and fertility under temperature stress** Kristen A Quaglia, Lisa N PetrellaMarquette University

As surface temperatures rise due to global warming, there will be catastrophic effects on the plants and animals that inhabit the Earth. A wide variety of organisms from rice and cattle to *C. elegans* will be impacted by the temperature sensitivity of their fertility. As temperatures increase, many organisms will face decreasing fertility and even sterility. We investigated how germline apoptosis may help to preserve fertility under temperature stress using 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. As temperature increases and fertility decreases, apoptosis in the germline increases over baseline levels. To understand how progeny fitness and fertility may be impacted by differing levels of apoptosis, we used 11 wild strains with varying fertility at the stress temperature of 26˚C to evaluate apoptosis and progeny fitness. If germline apoptosis increases to maintain fertility and progeny fitness during moderate temperature stress, we predicted that strains with higher levels of apoptosis will also have higher levels of fertility and their progeny will be more fit. Using two different measures of apoptosis, we found that apoptosis increased in some wild strains during temperature stress. We also evaluated measures of progeny fitness and found that under temperature stress lifetime fertility decreased, and embryo lethality increased in all strains, while some strains showed an increase in the size of fully cellularized oocytes. Our results indicated that apoptosis may have weakly affected fertility and progeny fitness as strains with higher levels of apoptosis tended to have higher fertility and lower levels of embryo lethality. Deciphering how *C. elegans* activate apoptosis to maintain fertility and progeny fitness under moderate

temperature stress is important for fully understanding how organisms can continue to produce high-quality, fit progeny despite stress conditions.

260B **CED-9-CED-4 Interaction is Likely Required for the Non-Canonical Pro-Apoptotic Function of CED-9** Nolan Tucker, Bob HorvitzHHMI/MIT

Apoptosis is a conserved process essential for proper development and tissue homeostasis in metazoans. In *Caenorhabditis elegans* the evolutionarily conserved process of caspase-mediated apoptosis is inhibited by CED-9. It has been proposed based on protein interaction and localization studies that CED-9 prevents cell death by physically interacting with and sequestering the pro-apoptotic protein CED-4 to mitochondria. Canonically, in cells fated to die EGL-1 (a BH3-only protein) binds CED-9 causing a conformational change in CED-9 that results in the release of CED-4, which then localizes to the perinuclear membrane where it activates the caspase CED-3. A strong *ced-9* loss-of-function mutation leads to maternal-effect lethality, presumably caused by excessive apoptosis since this phenotype can be suppressed by a loss-of-function mutation in *ced-4* or *ced-3,* both proapoptotic genes*.* In addition to its anti-apoptotic function, *ced-9* has a poorly understood pro-apoptotic function. For example, *ced-9(lf)* mutations can enhance the cell-death defect observed in animals with a weak loss-of-function mutation in *ced-3*.

The *ced-9* allele *n3377* is distinctive. In unpublished work (P. Reddien and H. R. Horvitz), *ced-9(n3377)* was isolated from a screen for enhancers of the cell-killing defect mediated by a weak loss-of function allele of *ced-3*, i.e. for mutations that decrease cell death. *n3377* carries an E74K missense mutation in the presumptive CED-4 binding pocket of CED-9, based on a crystal structure of a CED-9/CED-4 complex (Yan *et al*. 2005). In a wild-type background, *n3377* causes a cell-killing defect but not maternal-effect lethality, unlike *ced-9(0)* mutants, suggesting that *ced-9(n3377)* retains *ced-9'*s anti-apoptotic function but is mutant in *ced-9*'s pro-apoptotic function.

Using CRISPR-Cas9 I have isolated seven additional alleles of *ced-9* carrying distinct mutations in the CED-4 binding pocket. Like *ced-9(n3377)* mutants, animals carrying these alleles display ectopic survival of VC-like cells, indicating a defect in *ced-9*'s pro-apoptotic function. In a wild-type background, these mutants lack the maternal-effect lethal phenotype characteristic of *ced-9(0)* mutants*,* suggesting that, like *ced-9(n3377),* these mutations do not disrupt the canonical anti-apoptotic function of *ced-9.* These observations suggest that CED-9 interacts with CED-4 to drive its pro-apoptotic function.

If the loss of a CED-9/CED-4 interaction eliminates *ced-9*'s pro-apoptotic but not its anti-apoptotic function, then either *ced-9*'s canonical anti-apoptotic activity does not require a CED-9/CED-4 interaction or CED-9 and CED-4 can interact in two functionally distinct ways, one of which mediates *ced-9*'s anti-apoptotic activity and one of which mediates *ced-9*'s pro-apoptotic activity.

261B **Endomitosis of the *C. elegans* intestine is controlled by transcriptional downregulation of cytokinesis genes** Ramon Barrull-Mascaro, Lotte van Rijnberk, Matilde GalliHubrecht Institute

Development of multicellular organisms relies on precisely timed cell divisions to form specialized organs and tissues. During differentiation, the expression of cell cycle genes is often altered to achieve tissue-specific division patterns. Maturing cells can transition to non-canonical cell cycles, in which they skip classical phases of growth and division. The *C. elegans* intestine undergoes three distinguishable cell cycle variations: during embryogenesis, the precursor E cell undergoes canonical cell cycles to form the intestine; during larval development, most intestinal cells become binucleated and polyploid by undergoing endomitosis, a cell cycle variation in which the M-phase ends prematurely and results in a failure of cytokinesis, followed by multiple endoreplication cycles to increase their final ploidy. While the mechanisms governing endoreplication have been largely elucidated, how cells initiate and execute endomitosis cycles is poorly understood. Using live imaging, we show that intestinal cells undergoing endomitosis lack a central spindle and do not initiate cytokinetic furrowing. Single-molecule FISH and RNA-sequencing of purified intestinal cells revealed that many mitotic genes become downregulated during endomitosis, including most genes implicated in cytokinesis. By artificially forcing cells into additional rounds of M phase at different moments in development, we find that intestinal cells lose the capacity to undergo cytokinesis during late embryogenesis, as they mature to form a functional intestine. We are currently investigating how cells are able to specifically repress cytokinesis genes during intestinal differentiation, by analysing chromatin landscapes from purified intestinal cells using ChIC-seq. Together, our work uncovers how cells are able to inhibit cytokinesis during endomitosis, and sheds light into how tissue specific cell-cycle variations arise during development.

262B **Elucidating the role of RAB-10 in EGFR signaling during *C. elegans* vulva development** Clare FitzPatrick, Christian RocheleauAnatomy and Cell Biology, McGill Unversity

Epidermal Growth Factor Receptor tyrosine kinase (EGFR) signaling is important to many developmental processes in animals such as growth, survival, proliferation, and differentiation of cells. EGFR signaling is upregulated across many cancer types, such as metastatic colorectal, non-small-cell lung, breast, pancreatic, neck, and glioblastoma cancers, making it a common target for

therapeutics. Resistance to tyrosine kinase inhibitors is becoming increasingly common and is a growing issue in treating EGFR positive cancers. In the nematode *C. elegans*, EGF receptor (LET-23) signaling is integral to proper vulva development. Binding of the LET-23 ligand (LIN-3) activates a signaling cascade involving RAS/MAPK/ERK signaling. Suppression of anything in this pathway leads to a vulvaless phenotype, and overexpression leads to a multivulva phenotype. LIN-3 is secreted to the vulval precursor cells (VPCs) from the overlaying gonad, thus, basolateral localization of LET-23 is essential for proper vulva morphogenesis. The LIN-2/LIN-7/LIN-10 pathway is acts as a positive regulator of EGFR signaling in *C. elegans* vulva development by promoting basolateral localization of LET-23. Our lab has previously described an AGEF-1/ARF-1/AP-1 pathway that acts as a negative regulator of LET-23 basolateral localization. Loss of this pathway leads to too much LET-23 signaling and a multivuval phenotype. We have demonstrated that RAB-10 GTPase is required for the mislocalization of LET-23 in *agef-1* mutants. Furthermore, RAB-10 is required for the negative effects on LET-23 signaling from AGEF-1 and ARF-1, but not for AP-1. This led us to hypothesize that RAB-10 is interacting on the level of AP-1. Preliminary data suggests that LET-23 signaling and overexpression of RAB-10 negatively regulate AP-1. As well, LET-23 signaling may upregulate RAB-10. We hypothesize that EGFR promotes its own basolateral localization via activation of the RAB-10 GTPase, which in turn inhibits AP-1 as part of a positive feedback loop during *C. elegans* vulva development.

263B **Planar-polarized plasma membrane compartments in body wall muscle cells** Elise Cheynet, Sandra Duperrier, Alice Peysson, Amandine Chambert-Loir, Thomas BoulinMeLiS, CNRS UMR 5284, Université Lyon 1

The plasma membranes of cells display high levels of structure and organization. For example, ion channels are targeted to specific membrane sub-compartments (e. g., axon initial segment or synapses in neurons, intercalated discs in cardiomyocytes, apico-basal compartments in epithelial cells). Precise targeting gives such compartments distinct functional properties.

By studying the localization of potassium channels in *C. elegans* body wall muscles, we have discovered an entirely unsuspected case of membrane compartmentalization and planar cell polarity. Indeed, we have found that TWK-28 channels are enriched at the anterior tip of muscle cells, while SLO-1 channels are only found in the posterior half of each cell. In addition, proteins of the Dystrophin complex that are required for the surface expression of TWK-28 and SLO-1, also display asymmetric subcellular localizations.

We thus seek to understand the cellular and molecular mechanisms controlling the highly structured and polarized organization of the worm's sarcolemma.

We first used a candidate gene strategy, and found that DSH-1/Disheveled, the receptor CAM-1/Ror and the Wnt ligand EGL-20 are required to ensure muscle cell polarity. Next, we performed an unbiased visual screen using the localization of sarcoglycan as a readout. It allowed us to recover both mutants affecting (i) the polarity of muscle cells and (ii) the biosynthesis and subcellular localization of sarcoglycan itself. In addition to known effectors, we found three new genes affecting polarity, and 7 mutants with various defects in protein levels and organization. Using whole genome resequencing, we already identified two conserved kinases that regulate the distribution of sarcoglycan at the muscle surface. In addition, while null mutants of DYC-1 (a dystrophin-associated protein) strongly decrease sarcoglycan surface expression, we discovered that a specific class of *dyc-1* alleles alters polarity. This unexpected dual role of DYC-1 suggests a possible link between the processes that control planar cell polarity and protein content at the cell surface.

This novel example of planar cell polarity in a tractable genetic model organism may provide valuable insight into the molecular and cellular mechanisms that regulate cellular organization, allowing specific functions to be compartmentalized within a single cell.

264B **The conserved role of BYN-1/Bystin in cellular uptake and clearance in the *Caenorhabditis elegans* germline** Hyemin Min, Emily L Spaulding, Catherine S Sharp, Esther Jeon, Lyn S Miranda Portillo, Dustin L UpdikeThe Mount Desert Island Biological Laboratory

The molecular events that regulate cellular uptake and clearance are of fundamental importance to cell biology, animal development, and cancer. Here we show that GLH/Vasa helicases, which promote germline development and fertility across species, have an unlikely role in cellular uptake and clearance in the *C. elegans* germline. To find GLH-1 binding partners, we analyzed potential interactions through a yeast-two-hybrid screen and identified BYN-1, the homolog of human Bystin/BYSL. In humans, Bystin promotes cell adhesion and invasion in gliomas, and with its binding partner Trophinin, triggers embryonic implantation into the uterine wall. *C. elegans* embryos do not implant and lack a homolog of Trophinin, but both Trophinin and GLH-1 share unique and extended phenylalanine-glycine (FG)-repeat domains that enhance the Bystin/BYN-1 interaction. Endogenous BYN-1 is primarily nucleolar and partitioned away from cytoplasmic germ granules. However, BYN-1 enters the cytoplasm during the brief window of spermatogenesis to colocalize with GLH-1, and this interaction facilitates the clearance of residual bodies (RBs) by the overlying somatic gonad. We demonstrate that BYN-1 acts upstream of CED-1 to drive RB engulfment, a process

that relies on the FG-repeat domains of GLH-1 and GLH-2. These results show an evolutionarily conserved pathway where cellular uptake and engulfment are triggered through cytoplasmic localization of Bystin/BYN-1 to interact with FG-repeat domain proteins. Insights from this model system to study Bystin/BYN-1 likely extend beyond RB clearance and embryonic implantation to interactions during cell adhesion, invasion, and migration in the context of both tumorigenesis and development.

265B **Aurora A functions in a time-dependent manner to polarize *C. elegans* embryos** Bailey N de Jesus[1], Nadia I Manzi[2], Daniel J Dickinson[21]Glow Worms, The University of Texas at Austin, [2]Molecular Biosciences, The University of Texas at Austin

Cell polarity refers to asymmetrical distribution of molecules and organelles in the cell, which is critical for proper asymmetric cell division and other functions of almost all cell types. Polarity is regulated by the highly conserved partitioning defective (PAR) proteins, and the *C. elegans* zygote establishes anterior-posterior polarity by segregating anterior and posterior PARs to their respective domains. Recently, it was found that the cell cycle kinase Aurora A (AIR-1 in *C. elegans*) is required for the formation of a single polarized domain in a proper and timely manner. Embryos depleted of AIR-1 using RNAi predominantly form two posterior-like domains (marked by PAR-2), referred to as a bipolar phenotype. However, it remains unclear how AIR-1 contributes to proper PAR-2 membrane localization and/or membrane binding. To gain insights on how AIR-1 regulates PAR-2 dynamics, we first inhibited AIR-1's catalytic activity using a small molecule Aurora A inhibitor. Interestingly, we observed that inhibiting AIR-1 catalytic activity prevents PAR-2 from loading on the membrane, a phenotype that is distinct from AIR-1(RNAi). We hypothesized that the difference between acute AIR-1 inhibition (using drugs) and chronic AIR-1 depletion (by RNAi) might reflect different roles of AIR-1 in the germline vs. early embryo. To test this hypothesis, we depleted AIR-1 at different stages of oogenesis using the auxin-inducible degron (AID) system. Embryos that were depleted of AIR-1 using AID or RNAi prior to meiosis I exhibited bipolarity, but depleting AIR-1 just prior to polarity establishment (at the end of meiosis II) prevented PAR-2 from loading onto the membrane, similar to AIR-1 inhibition. Altogether these data indicate that AIR-1 functions in a time-dependent manner to regulate PAR proteins during *C. elegans* zygote polarization.

266B **CED-12, the original ELMO (engulfment and cell migration) protein, promotes F-actin during engulfment and inhibits it during cell migration** Thejasvi Venkatachalam, Martha SotoRutgers - RWJMS

ELMO (engulfment and cell migration) proteins were first discovered in *C. elegans*, and are conserved from humans to plants, fungi and amoebae. The ELMO family includes shorter ELMOD proteins, primarily composed of an ELMO domain, that typically encode a GAP with hydrolysis activity towards Arf and Arl GTPases. Longer ELMO family proteins, with additional domains, support the GEF function of partner CED-5/DOCK-180 proteins, to activate Rac/Rho family GTPases. The original ELMO, CED-12, is longer, includes a PH membrane binding domain, and was thought to act, together with CED-5/DOCK180, to activate CED-10/Rac1 during engulfment and cell migrations. We therefore expected CED-5/CED-12 to behave as a GEF for CED-10/Rac1, during a cell migration essential for *C. elegans* embryogenesis, ventral enclosure, when a sheet of epidermal cells migrates to enclose other tissues. Reducing CED-12/CED-5 should decrease F-actin during this migration if it acts as a Rac GEF. Instead, loss of CED-12 or CED-5 led to increased F-actin formation, increased dynamics in the migrating cells, faster migrations, and embryonic lethality. However, these migrating epidermal cells assembled lower F-actin to engulf dying cells, and corpses disappeared more slowly, as was expected for loss of a CED-10/Rac1 GEF. To address how CED-12/CED-5 have two opposing effects on F-actin, in the same cells, during corpse engulfment and cell migration, we investigated if CED-12 harbors ELMOD and ELMO functions. A candidate GAP region in CED-12 faces away from the CED-5 GEF catalytic region. Mutating a candidate catalytic Arginine in the GAP region (R537A) altered the epidermal cell migration function, and not the corpse engulfment function. Thus, a single ELMO/ELMOD protein can use distinct domains to carry out two opposite functions. Additional experiments identify candidate upstream signals that may activate the GEF or GAP functions of CED-12, and candidate GTPase targets of the GAP function. This study proposes that a single protein has both GEF and GAP functions, that result in opposite effects on F-actin, to coordinate two events within the same cells.

267B **Actin capping protein, CAP-1, regulates actomyosin contractility and maintains syncytial germline architecture** Priti Agarwal[1], Shinjini Ray[2], Anat Nitzan[2], Francois Nedelec[3], Ronen Zaidel-Bar[41]Tel-Aviv University, [2]Tel Aviv University, [3]University of Cambridge, [4]Cell and Developmental Biology, Tel Aviv University

Force generated by actomyosin machinery is essential for cell and tissue morphogenesis as well as the physiological function of many tissues. One example is the syncytial germline of *C. elegans*, where inward contraction of a corset-like actomyosin structure lining the rachis balances the outward pulling force of germ cell membrane tension. Here, we report a role for the actin capping protein CAP-1 in regulating the level of actomyosin contractility the rachis corset. With an endogenous reporter we show that CAP-1 localized at the rachis, and its depletion or overexpression led to severe structural defects in the syncytial germline and oocytes. A 60% reduction in the level of CAP-1 caused a 2-fold increase in F-actin and non-muscle myosin II activity, and laser incision experiments revealed an increase in rachis contractility. Cytosim simulations of cytoskeletal activity with different molecular compositions pointed to increased myosin as the main driver of increased contractility following loss of actin capping

protein. Double depletion of CAP-1 and NMY-2/myosin or LET-502/Rho Kinase demonstrated that the rachis architecture defects associated with CAP-1 depletion require contractility of the actomyosin corset. Thus, we uncovered a physiological role for actin capping protein in regulating actomyosin contractility to maintain germline architecture.

268B **The unconventional physical chemistry of the first *C. elegans* oocyte actomyosin cortex** Arjun Narayanan[1], Victoria T Yan[2], Tina Wiegand[2], Frank Julicher[3], Stephan Grill[21]Physics, New York University Abu Dhabi, [2]Max Plank Institute Cell Biology and Genetics, [3]Max Plank Institute for Physics of Complex Systems

We, and the worms we study, both start our lives as a single cell - an oocyte that must begin to divide and develop. To do this, the oocyte must first fill the region just under its plasma membrane with a contractile and dynamic meshwork of actomyosin filaments. But how is this first actomyosin cortex put together from constituent molecules? We will describe the discovery that in *C. elegans* oocytes, actomyosin cortical assembly relies on the emergence of thousands of short-lived protein condensates rich in actin filaments, and filament nucleators. We extract empirical growth laws governing the chemical dynamics of these condensates. Remarkably, these growth laws show that, the condensates are test tubes that both host, and are shaped by, the same chemical reactions. That is, mass action kinetics are coupled to assembly kinetics within the condensates. We will describe how such chemical reactions that shape their own test tube allow the *C. elegans* oocyte to prevent runaway actomyosin filament nucleation as it rapidly assembles its first cortex. We will also describe how the empirical growth laws reveal novel principles of intracellular physical chemistry likely to be applicable across cell biology.

269B **Investigating differences in spindle assembly checkpoint strength in the early *C. elegans* embryo** Imge Ozugergin[1], Abigail Gerhold[21]Biology, McGill University, [2]McGill University

The spindle assembly checkpoint (SAC) is a conserved mitotic regulator that preserves genome stability by inhibiting the anaphase-promoting complex (APC) and delaying anaphase onset until all chromosomes are attached to the mitotic spindle. Despite its central role in preventing chromosome segregation errors, variation in SAC strength is widespread, but poorly understood. Notably, early embryonic cells generally have a weak checkpoint, suggesting developmental regulation. In the *C. elegans* embryo, changes in SAC strength are associated with cell size and cell fate. SAC strength increases as cell size decreases due to cleavage divisions, and germline-fated cells have a stronger SAC than similarly sized somatic cells. To better understand what underlies these differences, we have taken a live-cell imaging-based approach to characterize the expression levels, localization and dynamics of endogenous, GFP-tagged mitotic proteins, including the core SAC proteins MDF-1/Mad1, MDF-2/Mad2, BUB-3, SAN-1/Mad3, over the first 5 rounds of embryonic cell division. We have developed image analysis tools to track and segment nuclei/chromatin throughout the cell cycle, to automatically define nuclear, cytoplasmic and chromatin-associated regions of interest and to measure fluorescence intensities within them. We can then associate these measurements with known differences in SAC strength. We found that expression of several SAC proteins, including MDF-2/Mad2, increase during this window of development, likely via *de novo* translation of maternally supplied transcripts. While expression of an APC subunit (MAT-2/ANAPC1) also increases, the rate of increase is lower, a scenario that would favor SAC activity at later developmental stages. While this difference in protein expression may contribute to increased SAC strength as cell size decreases during development, it does not account for the differences in SAC strength between similarly sized germline and somatic cells. Overall, these results add to our understanding of developmental variation in SAC strength and may also be relevant to SAC activity in pathological states such as some cancers where SAC protein expression increases.

270C **Characterization of proteostasis factors in the context of cellular safeguarding** QINMING LI, Gizem Köse, Marcel StudtMolecular Cell Biology Unit, Institute of Cell and Systems Biology of Animals, University of Hamburg, Germany

Proteostasis is essential for accurate protein level regulation. The loss of proteostasis is widely regarded as a hallmark of cellular senescence and age-related diseases. Recent studies have suggested that proteostasis mechanisms play a central role in stem cell fate decisions. However, regulatory mechanisms and specific functions of proteostasis factors in cell fate maintenance and during cellular reprogramming are not fully undestood. To better understand the implication of proteostasis regulators during transcription factor-mediated reprogramming, we make use of 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 reprogramming barrier genes, we identified the ubiquitin ligase-encoding genes *hecd-1, eel-1, math-33, marc-4,* and *usp-39*, which encodes a ubiquitin-specific protease, as barriers for germ cell into neuron-like cell reprogramming. Depletion of these factors by RNAi or CRISPR/Cas9-mediated gene knock-out consistently induces ASE neuron fate reporter expression and morphological changes of germ cells. We are assessing neuron-type specific gene expression also at the protein level and morphological changes to determine the extend of germ cell reprogramming to neuron-like cells. Furthermore, we also plan to apply Mass-spectrometry to monitor changes in the proteome upon depletion of the identified proteostasis factors. Our ultimate goal is to determine the context of proteostasis-regulating pathways during germ cell fate protection and TF-induced reprogramming of germ cells.

271C **CED-6/GULP and the AP2 complex maintain CED-1 localization on the plasma membrane via Clathrin-mediated**

endocytosis in *C. elegans* Rikke Harders[1], Tine Morthorst[2], Line E. Andersen[2], Anna D. Lande[2], Stine B. Mortensen[3], Bartosz Laczek[3], Anders Olsen[31]Chemistry and Bioscience, Aalborg University, [2]Aarhus University, [3]Aalborg University

The CED-1 protein is a transmembrane receptor homolog to the human SREC and CD91/LRP protein normally distributed in the plasma membrane. CED-1 is involved in the recognition of "eat-me" signals displayed on the surface of apoptotic cells and the subsequent engulfment of the cell corpse in *C. elegans*. How CED-1 mediates engulfment, has been intensively studied and the downstream effectors are well established. Some of these effectors are CED-7, which mediates the recognition of apoptotic cells and CED-6, which acts as an adaptor protein that binds directly to CED-1. When the apoptotic cell has been removed properly by engulfment, CED-1 is recycled to the plasma membrane by the retromer complex proteins SNX-1 and SNX-6. However, the mechanisms maintaining the CED-1 protein localization in the plasma membrane as long with its general internalization and recycling when not engaging in engulfment are currently unknown. Here we show that CED-6 and CED-7 are novel mediators in ensuring correct localization and endocytosis of CED-1, as depletion of either of the proteins together with subunits of the AP2 complex or clathrin results in mislocalization of CED-1 in the gonadal sheath cells. The mislocalization is seen when tagging the CED-1 protein with GFP as small distinct puncta resembling stalled CED-1 and therefore aggregation of CED-1. Depletion of proteins involved in endocytic sorting and recycling in *ced-6* mutants did not cause mislocalization of CED-1 proposing that CED-6 and CED-7 function in the early stage of CED-1 endocytosis. These findings reveal that CED-7 and CED-6 are involved in securing correct localization of CED-1 prior to engulfment of apoptotic cells.

272C **Exploring how cell cycles are timed in development using the *C. elegans* intestine as a model** Sonia Veltkamp, Matilde GalliHubrecht Institute

Animal development depends on precisely timed cell divisions, which ensure the formation of tissues and organs with specific architectures and functions. It is largely unknown how cells coordinate their cell cycle timings with developmental progression and whether, for example, developmental signals impinge on the cell cycle machinery to ensure timely divisions. In this project, we use the *C. elegans* intestinal lineage to study how cell cycles are timed during development. Intestinal cells sequentially transition through three distinguishable types of cell cycles at defined moments in development: in embryogenesis, intestinal cells undergo canonical cell cycles to form a functional intestine; during the L1 stage, intestinal cells undergo one round of endomitosis that gives rise to binucleated cells; subsequently, intestinal nuclei increase their ploidy by undergoing endoreplication cycles at the end of each larval stage. We have generated a strain with a fluorescent cell cycle marker (CYB-1DB::mCherry) and a molting reporter (Pmlt-9::GFP) to simultaneously visualize intestinal cell cycles and developmental timing. Using a recently developed microfluidics device (S. Berger et al., 2021), we can now track intestinal cell cycles and developmental progression over multiple larval stages. By combining this platform with intestine-specific degradation of cell cycle regulators, we plan to investigate how intestinal cell cycle timings are controlled in development. Specifically, we aim to degrade the cell cycle regulators CKI-1 and CYD-1 to alter the length of the G1 phase in L1 intestinal cells and study how this affects subsequent cell cycle timings. These experiments will elucidate whether intestinal cell cycles are coordinated with developmental time by a cell-intrinsic cell cycle timer or by responding to instructive developmental signals at defined moments in development. Our experimental design can be extended to investigate other cell cycle perturbations and provide deeper insights into the mechanisms governing cell cycle timing in development.

273C **The *C. elegans* intestine and germline share their small membrane-impermeable molecules** Sarah Turmel-Couture, Lucie Beaulieu, Pier-Olivier Martel, Patrick NarbonneUniversité du Québec à Trois-Rivières

Extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK\MAPK) is a positive regulator of cell proliferation that is often upregulated in cancer. In *C. elegans*, its ortholog MPK-1 stimulates germline stem cells (GSCs) cell non-autonomously, from the animal's intestine or somatic gonad. How MPK-1 performs this task however, from either of these two neighboring tissues, is a question that remains unresolved.

Notoriously, gap junctions interconnect the gut to the somatic gonad, and the somatic gonad to the germline while these junctions are essential for GSC proliferation. We therefore hypothesized that MPK-1 activity could lead to the diffusion of pro-proliferative small molecules through gap junctions, from the intestine or the somatic gonad to the GSCs.

To test this hypothesis, we experimentally established the diffusion pattern of a small membrane-impermeable fluorescent molecule, 5-carboxyflurescein (5-CF), following its micro-injection into the animal's intestine. Our results demonstrate that 5-CF transfers from the gut to the germline and vice versa, but does not wander into other tissues such as muscles or hypodermis. Also, small variations in the intestinal injection site, despite slightly affecting intra-intestinal dye diffusion, do not noticeably impact the flow to the germline. This demonstrates the possibility of a specific gut-to-germline signaling axis mediated by small membrane-impermeable molecules diffusing trough gap junctions. Interestingly, we noticed that the 5-CF diffusion pattern differs in some strains in which GSC proliferation is perturbed, but that these different diffusion patterns were independent from

GSC proliferation levels. We also noticed a preferential diffusion towards the proximal oocytes, some of which had turn into eggs, as compared to the pachytene region of the germline. We demonstrate that this preferential proximal 5-CF distribution is dependent on the endocytosis pathway that loads oocytes with yolk proteins. Further analysis should allow us to firmly establish whether small membrane-impermeable molecules could regulate GSCs downstream of MPK-1 signaling.

274C **Regulation of Kinesin-2 Motors in Sensory Cilia of *C. elegans*** Wouter Mul, Aniruddha Mitra, Erwin J.G. PetermanVrije Universiteit Amsterdam

Most eukaryotic cells contain a primary cilium, which acts as an antenna to sense the environment and is involved in a wide range of signalling pathway essential for development. Cilia consist of a microtubule-based axoneme that acts as a track for intraflagellar (IFT) transport of proteins back and forth along the cilium, necessary for correct ciliary functioning. In *C. elegans*, anterograde IFT trains, move from ciliary base to tip by the cooperation between two kinesin-2 motors, kinesin-II and OSM-3. The slower kinesin-II motors navigate the IFT trains through the densely-packed transition zone and proximal segment, handing over the IFT trains to the faster OSM-3 motors for transport further along the cilium. The regulatory mechanism that controls the cooperation between kinesin-II and OSM-3 remains elusive, although previous studies suggest certain kinases, DYF-5 and DYF-18 (RCK kinase and CCRK orthologs) play a crucial role. Here, we perform quantitative fluorescence microscopy in mutant *C. elegans* strains that lack the DYF-5 kinase, in order to understand how these kinases affect kinesin-2 motor cooperation. We find that the cilia of dyf-5 knockout (KO) animals are longer with a dramatic variability in length. In addition, kinesin-II is distributed differently along the cilium: in the mutant it is present throughout the cilium, while in wild-type animals it localizes to the beginning of the proximal segment. Moreover, in the mutant, we find that kinesin-II often accumulates at the tip and halfway the cilium. Next, we looked into the single-molecule dynamics of kinesin-II in WT and dyf-5 KO animals. We observed that the velocity of the individual kinesin-II motors is reduced in the absence of DYF-5 and that motors pause at the accumulation sites. These results provide mechanistic understanding in the role of kinases in kinesin-2 motor regulation.

275C **The distinct response of *C. elegans* chemosensory neurons to different repellents** Guus Haasnoot, Christine Bruggeman, Erwin PetermanPhysics and Astronomy, Vrije Universiteit Amsterdam

The nematode *C. elegans* has a relatively simple nervous system of only 302 neurons, 32 of which are specialized neurons able to sense the chemical environment. These neurons can sense attractive and/or repulsive ques and use this information to change their behaviour accordingly, a very useful feature to aid survival. Two pairs of chemosensory neurons are located in the tail of the worm and are denoted PHA and PHB. Through an opening in the cuticle, the ciliated ends of these neurons are exposed to the environment. Therefore, they are able to sense the surrounding chemical composition through receptors located in the membrane. Inside the cilium, intraflagellar transport (IFT) maintains the structure of the organelle and transports sensory proteins to the tip (anterograde) and back (retrograde). However, how exactly this transport system is regulated and how its regulation relates to neuronal signalling is not yet known. To investigate this, we use a microfluidic chip to stimulate the tail of the worm with aversive chemicals at high temporal resolution. We image the change in calcium concentration in our neurons of interest, which is a measure for neuronal activity, by using a calcium indicator. With this microfluidic chip we also study different IFT components which have been fluorescently labelled. During exposure to chemicals repellents we observed a redistribution of IFT components towards the base of the cilium as a result of the inhibition of anterograde transport. However, when we expose the worm to a hyperosmotic repellent, IFT particles start to accumulate at the tip of the cilium as a result of the inhibition of retrograde transport. To obtain more mechanistic insight into this change in IFT dynamics we recently developed a method to study the IFT components on the single-molecule level while exposing the worm with an aversive chemical.

276C **Uncovering how heterochronic factors control intestinal cell cycle progression in development** Daniel Iglesias van Montfort, Matilde GalliHubrecht Institute

In order for cells to form functional tissues and organs, they must divide at specific times and and coordinate their divisions during development. How cells time their cell cycles during development is largely unknown, however research from the past decades has uncovered a group of genes called “heterochronic genes” that control cell division timings in *C. elegans*. One of these, *lin-14*, encodes a transcription factor that is required for cells to undergo the particular division patterns that occur during the first larval stage (L1). At the end of L1, *lin-4*-mediated *lin-14* mRNA degradation allows cells to transition to L2-stage cell fates. In *lin-14* loss-of-function mutants, cells skip the L1 stage division patterns and initiate the L2 stage division patterns directly after hatching. It is currently unknown how LIN-14 controls cell division patterns, and thus, if it controls the expression of cell-cycle regulators. In this project, we make use of the intestinal lineage, which undergoes three distinguishable cell cycle variations during development: (1) during embryogenesis, intestinal cells undergo canonical cell cycles to form a functional intestine; (2) during the L1 stage, intestinal cells undergo one round of endomitosis that gives rise to binucleated cells; (3) subsequently, intestinal nuclei increase their ploidy by undergoing endoreplication cycles at the end of each larval stage. Using heat shock-inducible *lin-4* expression we can target *lin-14* mRNA degradation during different moments in L1 and determine the effect

on intestinal cell-cycle progression. As expected, we find that overexpression of *lin-4* during early L1 results in intestinal cells skipping endomitosis and remaining mononucleated, similar to *lin-14* loss of function mutants. Interestingly, our preliminary results suggest that *lin-4* overexpression does not inhibit binucleation during later timepoints in L1, when intestinal cells are likely in G2 phase of endomitosis, suggesting that at that stage intestinal cells have become committed to undergo endomitosis. In future work, we plan to use a microfluidics-based approach to image endogenously GFP-tagged LIN-14 and make use of the Auxin-Inducible Degron (AID) system, to target LIN-14 degradation during specific cell cycle phases in intestinal cells, which will allow us to further investigate how LIN-14 dynamics control intestinal cell-cycle progression. Furthermore, we plan to perform RNA-sequencing on purified intestinal cells in the presence and absence of LIN-14 to investigate how LIN-14 influences cell-cycle gene expression. Together, this work will help elucidate the mechanisms by which heterochronic factors impinge on the cell cycle machinery, which will be critical to understand how cells time their divisions during development.

277C **Comparison of meiotic proteins REC-1/HIM-5 in *Caenorhabditis elegans* and *Caenorhabditis briggsae*** Michelle Scuzzarella, Judith L. YanowitzOBGYN, Magee-Womens Research Institute

DNA double-strand breaks (DSBs) are highly deleterious, yet necessary for exchange of genetic material during crossover (CO) formation in meiosis. CO formation is required for the physical connection and proper alignment of homologs to prevent nondisjunction of chromosomes during meiosis I.

In *C. elegans,* most chromosomes receive a single CO on the recombinogenic and less gene-dense chromosome arms, while avoiding the more gene rich chromosome centers. In this organism, the *rec-1* gene is responsible for maintaining normal CO distribution. The number of CO events remains unchanged in *rec-1* mutants, while the location of crossovers is altered. The *him-5* gene plays a role in maintaining normal crossover distribution, while also promoting DSB formation on the X chromosome. When both genes are mutated, more severe defects in DSB formation are observed with nondisjunction of autosomes also occurring.

Prior studies showed that the *rec-1* and *him-5* genes are distantly related paralogs with a single ancestral gene in other *Caenorhabditis* species. The gene coding for REC-1 is in a synteny block on LG I that is conserved in order and orientation in at least six other *Caenorhabditis* species but the genes in the *rec-1* position show greater sequence similarity with *C. elegans him-5*, which is found on LG V. This evidence, as well as their redundant roles in meiosis suggest that *rec-1* and *him-5* are distantly related paralogs.

We have used the CRISPR/Cas9 gene editing system to create a knockout mutant in the *Caenorhabditis briggsae* paralog of these two genes, *cbg25171*, with the intention of exploring the evolution of these genes and the effect on meiosis in a closely related species. The presence of males in this population has been observed, as well as an increased number of univalents at diakinesis, which indicates an increase in nondisjunction. Based on preliminary data, we hypothesize that *cbg25171* plays a similar role in *C. briggsae* meiosis as both *rec-1* and *him-5* do together in *C. elegans* despite low sequence similarity. Further functional analysis of the *C. briggsae* mutations of the *rec-1/him-5* paralog will be presented.

278C **Tryptophanyl tRNA synthetase (WARS1) depletion leads to genomic instability** Mahmoud Izadi, Tayyiba Akbar Ali, Farah Shurrab, Ehsan Pourkarimi D.Division of Genomics and Translational Medicine, College of Health and Life Sciences, Hamad Bin Khalifa University

The highly conserved aminoacyl tRNA synthetases (Aars) are, at a superficial glance, housekeeping genes essential for catalyzing the binding of amino acids to their cognate tRNA, ensuring translation fidelity. Aars also have non-canonical functions unrelated to their role in aminoacyl transferase activity. Recently, mutations in members of the Aars protein family have been reported in different cancers. Among them, tryptophanyl tRNA synthetase (WARS) has been related to an increased risk of metastasis. Previously, we identified the correlation between *wars-1* knockdown (KD) and improper cell division in the mitotic zone, leading to defects in germline development in *Caenorhabditis elegans* (*C. elegans*). Here, we aim to further characterize the effects of WARS-1 depletion on germline development. We report the effect of the *wars*-1 KD on genomic instability; worms with reduced WARS-1 exhibit cell cycle arrest at the G2/M phase transition of the mitotic cells. This cell cycle arrest phenotype is linked to the activation of the DNA damage checkpoint signaling, confirmed by CHK-1 phosphorylation. We further show that the DNA damage checkpoint activation is accompanied by increased RPA-1 foci in both mitotic and meiotic cells. Interestingly, *wars-1* KD in the mitotically active embryonic cells results in the formation of chromatin bridges, which is the hallmark of genomic instability or the lack of proper DNA damage repair. All in all, to our knowledge, we are the first to report the role of *wars-1* KD or perhaps the lack of proper amino acid loading and protein translation in genomic instability. This further suggests the protective role of WARS-1 in cancer formation and metastasis, making it a promising target for cancer treatment.

279C **Dinstint collaboratory Aurora B kinase and the PP1 phosphatases during the two *C. elegans* male meiotic divisions** YU-NAN TSAI, Yi-Shiu Lin, Jui-Ching WuClinical Laboratory Sciences and Medical Biotechnology, National Taiwan

University

Accurate and precise segregation of chromosomes is essential for maintaining chromosome integrity during cell division. It has been shown that collaboration between Aurora B kinase and PP1 phosphatases is essential for regulating chromosome bi-orientation and segregation in mitotic and oocyte meiotic divisions. In this study, we examined the roles of Aurora B kinase and PP1 phosphatases during male meiotic divisions. An analog-sensitive Aurora B kinase worm enables the examination of MI and MII divisions separately. As expected, inhibition of Aurora B kinase AIR-2 in meiosis I caused segregation defects including mis-orientation and nondisjunction. Similarly, inhibition of Aurora B kinase in secondary spermatocytes also caused chromosome segregation defects comparable to the inhibition of Aurora B kinase in meiosis I. As an antagonistic factor, we found PP1 phosphatase GSP-2 is required for both MI and MII chromosome segregation. Deletion of PP1 GSP-2 caused significant mal-alignment of chromosomes at metaphase onset in MI and subsequent MII. In contrast, primary spermatocytes treated with Pan-PP1 phosphatases inhibitor okadaic acid exhibited chromosome orientation defects comparable to *gsp-2* mutants, indicating GSP-2 is the major phosphatase during meiosis I. However, okadaic acid treatment induced a different set of chromosome segregation defects in male meiosis II, where chromosomes failed to align bi-orientally before separation, and nondisjunction also occurred. Thus, male MI and MII divisions might employ different phosphatases for regulation. Our study reveals that Aurora B kinase participates in regulating both MI and MII in a kinase/phosphatase-dependent phosphorylation with different PP1s, and proper coordination of the kinase/phosphatase balance is critical for accurate chromosome segregation during male meiosis.

280C **Regulation of *C. elegans* germline stem and progenitor cell mitosis by developmental and environmental signaling networks** Eric Cheng, Ran Lu, Abigail GerholdBiology, McGill University

Equal partitioning of the replicated genome is essential to produce genetically identical daughter cells during mitosis. How mitotic fidelity is affected by physiological changes in developmental or environmental signaling networks remains unclear, in part due to a lack of suitable model systems for the real-time observation of mitotic cells *in vivo. C. elegans* germline stem and progenitor cells (GSPCs) provide an excellent model system in which to investigate the influence of signaling networks on mitotic cells within their native environment. Using *in situ* live-cell imaging, we have shown that caloric restriction and developmental changes in GSPC proliferation rate are associated with a prolonged duration of GSPC mitosis, as measured from nuclear envelope breakdown to anaphase onset. Here we have performed a candidate screen of major developmental signaling pathways, which integrate cell proliferation and development in many organisms, but which are not known to influence mitosis *per se*, for similar effects on GSPC mitotic timing. We find that reducing activity of the insulin receptor *daf-2*/IGFR (*daf-2(e1370)*) leads to an increased duration of GSPC mitosis that is dependent on the mitotic checkpoint, and an increased incidence of chromosome segregation errors when the checkpoint is compromised. *daf-2(e1370)* mitotic delays can be rescued by loss-of-function mutations in *daf-16*/FOXO or *daf-18*/PTEN or a gain-of-function mutation in *akt-1*, suggesting that they require canonical insulin signaling, converging on the regulation of DAF-16. However, despite a measurable increase in DAF-16 nuclear localization in GSPCs in *daf-2(e1370)* mutants, we find that DAF-2 is required in the soma, and not in the germline, to affect both GSPC mitotic duration and cell cycle progression. In addition, although *daf-2(e1370)* phenocopies the effect of caloric restriction on GSPC mitosis, we find that mitotic delays upon caloric restriction require *daf-18*/PTEN, but not *daf-16*/FOXO, indicating that developmental and environmental signaling networks make distinct, genetically separable contributions to the regulation of mitosis in GSPCs. Overall, our work highlights the challenges cells face to preserve mitotic timing and fidelity in complex physiological environments.

281C **Homeostatic regulation of germline stem cells : AAK-1 non-autonomously supresses germline stem cell proliferation from the somatic sheath cells** Xavier Lechasseur, Olivier Gagné, Ange Brou, Patrick NarbonneMedical biology, Université du Québec à Trois-Rivières (UQTR)

Maintaining a balance between stem cell proliferation and the need for their differentiated progeny is crucial for all organisms. Breaking this balance can lead to tissue atrophy or to the development of tumors. To prevent such anomalies, homeostatic regulatory mechanisms couple these two variables *in vivo*. In *C. elegans*, homeostatic signaling blocks germline stem cell (**GSC**) proliferation when oocytes accumulate in the proximal part of the gonad because of sperm depletion. AAK-1, the *C. elegans* ortholog of the AMP-activated protein kinase (AMPK), is required for homeostatic downregulation of GSC proliferation. To understand this new AAK-1 role, we first determined in which tissue(s) it was required for homeostatic signaling. We tagged endogenous AAK-1 using CRISPR/Cas9 to find that it was highly expressed in the gonadal sheath cells and was also present at lower levels in the distal tip cell (DTC), intestine, and throughout the germline. We performed *aak-1(RNAi)* in the *rrf-1* and *ppw-1* backgrounds to inactivate *aak-1* specifically in the germline and soma, respectively. These experiments clearly showed that AAK-1 activity was required in somatic tissues for homeostatic signaling, and thus functions cell non autonomously to suppress GSC proliferation. Using tissue-specific rescuing transgenes to restore AAK-1 function in *aak-1(-)* mutants, we further determined that AAK-1 expression in the gonadal sheath cells was sufficient to rescue homeostatic signaling. Interestingly, the growth signaling effector MPK-1/MAPK promotes GSC proliferation within the same tissue, raising the possibility that AAK-1 could interact with MPK-1 signaling in the sheath cells to stop GSC proliferation. To genetically order this interaction, we constructed an *aak-1(-) mpk-1(-*

) double mutant. The phenotype of this double mutant was similar to the *mpk-1(-)* phenotype, suggesting that *aak-1* could act upstream to inhibit *mpk-1* during homeostatic signaling, as part of the same pathway. We are currently investigating whether this interaction may or may not be direct. In case the interaction would not be direct, we also are exploring in parallel the AAK-1 interactome with the Turbo ID labeling technique to find out, within the gonadal sheath cells, other proteins that should lie between AAK-1 and MPK-1 to mediate homeostatic signaling.

282C **TOP-2 is differentially required for meiotic chromosome morphology in spermatogenesis and oogenesis** Christine K Rourke[1], Nirajan Bhandari[1], Lauren Salvitti[2], Aimee Jaramillo-Lambert[11]University of Delaware, [2]Charles River Laboratory

The specialized cell division of meiosis results in the production of haploid gametes from diploid gamete precursor cells. The success of meiosis I is dependent on the proper pairing of homologous chromosomes, synapsis, and recombination. Failure to complete these steps properly results in gamete aneuploidy, which is the leading cause of infertility, progeny inviability, and birth defects. Many meiotic events are sex-specific including chromosome structure. In particular, the degree of chromosome compaction in spermatogenesis is much greater than in oogenesis. Differential chromosome compaction appears to be co-ordinated by the functions of several proteins including sperm-specific histone variants and histone post-translational modifications, condensins, cohesins, and DNA topoisomerases. Recently, we found that *C. elegans* Topoisomerase II (*top-2*) plays sex-specific roles in the localization of meiotic chromosome structural components and on chromosome structure during late meiotic prophase. During spermatogenesis, late prophase chromosomes are significantly compromised both in their ability to condense and to individualize after loss of *top-2* function [*top-2(it7)*]. During oogenesis, chromosome individualization and overall structure appears to be unaffected in *top-2(it7*), however, the length of diakinesis bivalents are elongated. These sex-specific differences are likely due to the temporal regulation of meiosis in late meiotic prophase of oogenesis vs. spermatogenesis. As *top-2(it7)* resulted in a change of chromosome morphology during late meiotic prophase of spermatogenesis, we asked if *top-2* is important for mediating a chromosome structure conducive to meiotic recombination. We examined meiotic double-strand break repair by the assembly and disassembly of RAD-51 foci during spermatogenesis. We found fewer RAD-51 foci in the transition zone through late pachytene in *top-2(it7)* spermatogenic germlines. Even though fewer RAD-51 foci are observed, crossover designation does not appear to be perturbed in *top-2(it7)* spermatogenesis as five GFP::COSA-1 foci were found per nucleus in the mutant germlines (one focus for each pair of autosomes, no focus on the X). Currently, we are further probing the spermatogenesis-specific roles of TOP-2 by investigating if *top-2(it7)* induces changes in the genetic map, and using a combination of FISH oligopaint and super-resolution microscopy to analyze chromosome structure during the karyosome to diakinesis transition.

283C **TIR-1/SARM1 is required for homeostatic regulation of germline stem cell proliferation independently of PMK-1/p38 MAPK** Alexandre Clouet, Matthieu Valet, Benjamin Dufour, Janina Rieger, Alexane Murray, Patrick NarbonneDépartement de Biologie Médicale, Université du Québec à Trois-Rivières

All differentiated cell types populating tissues and organs derive from stem cells. To maintain tissue and organ homeostasis, stem cell proliferation must be finely tuned during the whole life of an organism to prevent developmental disorders and pathologies like cancers. In *C. elegans*, germline stem cell proliferation rates are regulated by two factors: nutrient uptake and the quantity of mature oocytes. In the latter, oocyte accumulation promotes germline stem cell quiescence, preventing oocyte hyperaccumulation. This homeostatic feedback needs to integrate oocyte abundancy and to signal across multiple tissues to modulate germline stem cell proliferation. Although we have identified DAF-18/PTEN, PAR-4/LKB1, AAK-1/AMPK and MPK-1/ERK as effectors of this negative feedback loop, additional effectors are presumably missing to complete this inter-tissular molecular cascade. Here, we EMS-mutagenized an *oma-1; oma-2* background to trigger oocyte accumulation and homeostatic inhibition of germline stem cell proliferation. We further used a germline marker to screen for mutants that displayed oocyte hyperaccumulation and phenocopied *aak-1; oma-1; oma-2* homeostatic defective mutant. We screened approximately 8000 haploid genomes from which we isolated 9 verified candidates. Using whole genome sequencing and bioinformatics analysis, we identified 800 genes that had mutations in these candidates, while 26 of these genes were mutated in two or more candidates. Complementation tests and transgenic rescue experiments confirmed that *aak-1* loss-of-function was responsible for the tumorous phenotype observed in two candidates. Hence, our screening strategy effectively led to the isolation of mutations disrupting homeostatic signaling. We next used RNAi to knockdown the remaining 25 genes on our shared list and identified TIR-1/SARM1 as a new gene required for of homeostatic signaling. Available null alleles further confirmed this *tir-1* phenotype. We next C-term tagged all endogenous TIR-1 isoforms using CRISPR/Cas9. TIR-1 expression was detected in nervous system and somatic gonad sheath cells. We next performed loss-of-function experiments on TIR-1's putative interactors. Among these, the knockout of PMK-1/p38 MAPK did not impair homeostatic signaling, suggesting that TIR-1 functions independently from this well-established target. Describing TIR-1 tumor suppressing function at a molecular level is important, as this role appears to be conserved in human.

284C **High-resolution recombination mapping in individual meiotic products of *C. elegans* spermatogenesis and oogenesis** Zachary Bush[1,1], Alice S Naftaly[1,1], Devin Dinwiddie[1], Cora Albers[1], John S Conery[1], Kenneth J Hillers[2], Diana E Libuda[11]Biology,

University of Oregon, [2]Biological Sciences, California Polytechnic State University, San Luis Obispo

Crossover recombination events generate genetic diversity and ensure the repair of DNA damage and the accurate segregation of chromosomes during meiosis. In many species, the genomic distribution of crossovers is nonrandom and can vary between sexes. The genomes of many species evolved kilobase-scale "hotspots" where crossover events are more likely to form. In contrast, the *Caenorhabditis elegans* genome lacks hotspots, with crossovers instead occurring across megabase-scale domains on the terminal thirds of each chromosome. Further, genetic and cytological experiments indicate *C. elegans* spermatogenesis exhibits higher crossover frequencies in comparison to oogenesis, however, the mechanisms that lead to this sexually dimorphic crossover regulation are poorly understood. To define these sexually dimorphic recombination landscapes at high resolution across the entire genome, we recently generated new reference genomes for the genetically divergent Bristol and Hawaiian isolates of *C. elegans*. Between the Bristol and Hawaiian lineages, we identify over 3.1 Mb of sequence divergence consisting of 337,584 SNPs, 94,503 small insertion-deletions (<50bp), and 4,334 large structural variations (>50bp). We also comprehensively annotated all transposable element families in both genomes and tracked the movement of 47 individual transposons between the two isolates. Finally, we performed whole-genome sequencing and high-resolution recombination mapping of single worms with Bristol-Hawaiian recombinant chromosomes from individual meiotic products of either spermatogenesis or oogenesis. Our preliminary results confirm that crossovers in the oocyte and spermatocyte genomes are formed at a higher rate in the terminal domains of each chromosome. Also, we detect and analyze rare incidences of double crossovers, and noncrossover recombination events. Overall, these studies present both a comprehensive analysis of the genomic variation between Bristol and Hawaiian populations of *C. elegans* and high-resolution whole-genome analysis of sexual dimorphic crossover distributions in individual *C. elegans* spermatocyte and oocyte genomes. Our work here also provides additional resources for future comparative genomic studies and a framework for high-throughput recombination mapping in both oocyte and spermatocyte genomes.

285C **Polarity regulation through microtubules** Aaron Daniel Brooks[1], Jack Martin[2], Josana Rodriguez[2], John Packer[2], Iolo Squires[21]ICAMB, Newcastle University, [2]Newcastle University

Polarity in the *C. elegans* zygote is regulated by a conserved group of polarity effectors classified as partitioning defective (PAR) proteins. These PAR proteins form distinct domains within the zygote and are labelled as being either anterior or posterior depending which domain they occupy. They are highly regulated as downstream signalling from these domains is ultimately responsible for successful asymmetric division and development. Symmetry breaking, the initial polarisation step in the C. elegans embryo, is traditionally thought to be achieved through two functionally redundant pathways. Each of these pathways rely on the activity of cytoskeletal filaments and are triggered by signals emanating from the centrosomes. One pathway involves the removal of anterior PARs through cortical flow generated from the retraction of an actomyosin cortex. Simultaneously centrosomal microtubules facilitate the loading of posterior PARs onto the cortex through the other pathway. Of these two, our study focuses on the microtubule dependent pathway. This was achieved through the use of a strain in which cortical flow is abolished (NOP1Δ).

Current understanding tells us that in the absence of cortical flows, the microtubule dependent pathway should be solely responsible for symmetry breaking. To some extent our data supports this, as the point of symmetry breaking correlates more with centrosome position in the NOP1Δ, where the microtubule pathway will be active. However, *RNAi* silencing of proteins thought to be within this pathway have shown some surprising results. Such as symmetry breaking occurring even when both pathways are theoretically not active. Our results highlight key proteins as well as gaps in our current understanding of the microtubule dependent pathway. Additionally, our results could be indicating that there is a third novel mechanism at play, in which microtubules are actively removing or delivering PARs, for which we have preliminary evidence

286C **Regulation of defective mitochondrial DNA accumulation and transmission in *C. elegans* by the programmed cell death and aging pathways** Samantha Fiallo[1], Sagen E Flowers[2], Rushali Kothari[2], Yamila N Torres Cleuren[3], Melissa R Alcorn[2], Chee Kiang Ewe[2], Geneva Alok[2], Abhay Saini[2], Pradeep M Joshi[2], Joel H. Rothman[21]UC Santa Barbara, [2]UCSB, [3]University of Bergen

Produced by engulfment by a primordial eukaryotic cell over 2.5 billion years ago, mitochondria contain their own genomes (mtDNA) that are distinct from that of the nucleus. mtDNA is especially susceptible to damage by superoxide radicals. Smaller, deleted mtDNA genomes are at a replicative advantage and, if not eliminated, can lead to debilitation or lethality of the animal. Eukaryotic cells have therefore evolved the process of mitochondrial purifying selection (MPS), in which mitochondria with defective mtDNAs are selective removed. The exact mechanisms of MPS are still largely unknown. We availed of a stable 3.1 kb mtDNA deletion, *uaDf5*, to investigate the role of regulatory components in MPS during germline development. This mutant mtDNA is carried in stable heteroplasmy with normal mtDNA and is the dominant species of the mitochondrial genome, likely owing to replicative advantage. We found that mutants lacking pro-apoptotic regulators, including the CED-3 and CSP-1 caspases, CED-13/BH3-only domain, and cell death engulfment factors, resulted in a substantially higher fractional abundance

of *uaDf5* in the germlines of mothers. This finding implicates the cell death machinery in MPS. However, we were surprised to find that removal of the pro-apoptotic factor CED-4/Apaf1 or mutations in the CED-4 interacting prodomain of CED-3 did not increase *uaDf5* levels, suggesting a non-canonical germline programmed cell death mechanism involving the CED-13/caspase axis. Our preliminary findings suggest that the defective mtDNA results in elevated cell death in the germline and arrest of germline proliferation, reminiscent of what is seen in response to radiation-induced genotoxic stress in the nuclear genome. Further, we found that *uaDf5* levels increase in mothers as they age, an effect that is passed onto their progeny. We found that in mutants with elevated *uaDf5* levels, MPS was enhanced in older mothers, suggesting age-dependence of MPS. Consistent with this possibility, we found that long-lived *daf-2* and *clk-1* mutants showed decreased accumulation of *uaDf5*, whereas short-lived mutants showed an increase in the defective mtDNA. These studies implicate both a non-canonical programmed cell death mechanism and the aging program in MPS and mtDNA quality control.

287C **Uncovering the Molecular Mechanisms that Underlie *C. elegans* Primordial Germ Cell Trogocytosis** Julie E Manikas, Yusuff Abdu, Jeremy NanceNew York University School of Medicine

A specialized form of cell-cell interaction called trogocytosis occurs when one cell cannibalizes another cell by biting off and digesting pieces of the cell. We showed previously that primordial germ cells (PGCs) form cellular extensions called lobes which are bitten off and digested by surrounding endodermal cells. This event results in a two-fold reduction in PGC size and mitochondrial DNA. Trogocytosis requires F-actin and the membrane remodeling proteins dynamin and LST-4/SNX9, but additional proteins needed to promote trogocytosis are unknown. Specifically, how trogocytosis differs mechanistically from whole-cell phagocytosis, which does not require a biting step, remains unknown. To identify additional regulators of trogocytosis, we performed a forward genetic screen and identified mutants defective in PGC trogocytosis. By analyzing cell corpse phagocytosis, we found that a subset of the mutants is required for PGC trogocytosis but not phagocytosis. Using FRAP analysis, we showed that some mutants are required for the biting step, whereas other function downstream to digest the bitten off pieces of PGCs. The ongoing cloning and characterization of these mutants will provide new mechanistic insights into a largely unexplored and unique form of cell-cell interaction.

288C **Skp1 proteins are structural components of the synaptonemal complex in *C. elegans*** Joshua Blundon[1], Brenda Cesar[1], Jung Woo Bae[1], Ivana Cavka[2], Jocelyn Haversat[3], Simone Koehler[2], Yumi Kim[31]Biology, Johns Hopkins University, [2]European Molecular Biology Laboratory, [3]Johns Hopkins University

Faithful chromosome segregation during meiosis requires chromosomes to pair and recombine with their homologous partners during meiotic prophase I. In most eukaryotes, homologous chromosome alignment is reinforced by synapsis, a process defined by the assembly of the synaptonemal complex (SC), a tripartite protein structure that assembles between homologous chromosomes. The SC interacts with crossover-promoting factors and enables their diffusion and concentration along meiotic chromosomes, thereby regulating the number and distribution of crossovers. Recent evidence from diverse eukaryotes indicates that the Skp1-Cul1-F-box (SCF) E3 ubiquitin ligase regulates synapsis, although the mechanism of its action remains unclear. Here, we report that two paralogous Skp1-related proteins in *C. elegans*, SKR-1 and SKR-2, serve as structural components of the SC, independently of their canonical roles within the SCF complex. SKR-1 and SKR-2 associate with the other SC proteins and localize to the SC central region, constituting its central element. As recently shown for *Dictyostelium* Skp1, recombinant SKR-1 forms a dimer in vitro. Strikingly, mutating the dimer interface of SKR-1/2, without impairing the SCF activity, results in a complete failure in synapsis and crossover formation, which is indistinguishable from the phenotypes observed in worms lacking other SC proteins. Intriguingly, the Skp1 dimerization interface is predominantly hydrophobic and overlaps with the binding sites for Cul1 and F-box proteins. Thus, the dimerization of SKR-1/2 and SCF formation are mutually exclusive, providing a molecular basis for why SKR-1/2 cannot function as part of the SCF complex once incorporated into the SC. Together, our findings reveal a remarkable case of meiotic regulation where a highly conserved cell cycle regulator is repurposed as part of the essential meiotic scaffold and may provide a mechanism for coupling SC assembly and disassembly with cell cycle progression.

289C **Defining CLS-2 Function in Central Spindle Assembly in C. elegans Male Sperm Meiosis** Sebastian Gomez[1], Vanessa Cota[21]Biology, San Francisco State University, [2]San Francisco State University

Male infertility is a serious concern for many Americans. To elucidate how infertility arises, we are using the *Caenorhabditis elegans* nematode to study unique molecular mechanisms across mitosis, oocyte meiosis, and male sperm meiosis that ensure proper chromosome segregation. In the case of mitosis and oocyte meiosis, a central spindle structure composed of microtubules assembles in the midzone and produces pushing/pulling forces between segregating chromosomes. The central spindle structure is initiated by the kinetochore protein, CLS-2. In the case of *C. elegans* male sperm meiosis, few microtubules are present in the midzone except those tied to the lagging unpaired X chromosome, a unique feature of male sperm meiosis. It is unknown whether the presence of the lagging unpaired X chromosome implies that there is a distinctive mechanism of chromosome segregation in male sperm meiosis. I hypothesize that CLS-2 does not play a significant role in central spindle assembly in male sperm meiosis.

Instead, male sperm meiosis relies on the pulling forces of the kinetochore-connected microtubules rather than the push/pull forces generated by the central spindle. After conducting immunostaining experiments, I have discovered that in addition to CLS-2 localizing to kinetochores on DNA and centrosomes on the polar ends of a dividing sperm cell, CLS-2 also co-localizes with microtubules in the midzone region during anaphase I of male sperm meiosis which I believe is meant to stabilize the lagging X chromosome as it resolves to a polar end. We are investigating if this new localization pattern is consistent throughout the rest of male sperm meiosis. To observe the spatial/temporal relationship CLS-2 has with microtubules and DNA, I have created a new *C. elegans* strain that contains fluorescent markers on CLS-2, microtubules, and DNA. I have successfully depleted CLS-2 in live C. elegans males using the novel auxin-inducible degradation system and will be observing any differences in male sperm meiosis with respect to microtubules and DNA. Measuring rates of DNA movement will allow us to determine the role of CLS-2 in segregation dynamics. We also plan to observe whether removing the presence of the lagging X chromosome (like in the case of hermaphrodite sperm meiosis or tra-2 mutant worms) will have an influence on CLS-2 function. Understanding CLS-2 function can elucidate the molecular mechanisms required for forming healthy sperm.

290C **Leveraging the Male Secreted Short (MSS) family of glycoproteins to investigate fertilization and sperm competition in *Caenorhabditis*** Asan Turdiev, Eric S. HaagDepartment of Biology, and Biological Sciences Graduate Program, University of Maryland, College Park

Sperm competition is a major component of sexual selection and is present in all major animal groups. Research from the past decades on several animal models, including flies, mice, and nematodes, has led to the identification of genes with apparent roles in sperm competitiveness [1, 2]. Their molecular mechanisms are generally not known, but they appear to be distinct from core fertilization factors. The dispensable nature of sperm-competition genes for reproduction makes them more amenable for manipulation than essential fertilization genes, yet they may reveal general lessons about gamete biology. Using comparative genomics, the Haag lab previously identified genes encoding small sperm glycoproteins that are present in outcrossing species, but consistently lost in hermaphrodites. These comprise the Male Secreted Short (MSS) family [3]. Restoring *mss* to males of the self-fertile *C. briggsae* via transgene from a close male-female relative *C. nigoni* led to a large increase in male mating success in competitive contexts. MSS is a short glycoprotein present on the surface of activated sperm. Its relatively well-conserved N-terminal signal peptide and C-terminal GPI anchor signal sequences are cleaved post-translationally, leaving a poorly conserved glycosylated region. My work seeks answers to three questions: Is glycosylation essential for the increased competitiveness of MSS+ sperm? Do other MSS-related proteins (MSRPs) perform distinct roles in *Caenorhabditis* sperm? What factors are responsible for *mss* expression in species that lost the gene? I hypothesize: 1) that increased sperm competitiveness is driven by MSS-conjugated glycans, which interact with sugar-binding receptors (e.g. C-type lectins) on female reproductive tissues; 2) that MSRPs are retained because they are required (alone or in combination) for baseline fertility, and 3) an evolutionarily conserved transcription factor is responsible for *mss* expression in species that lost *mss*. I will report recent results that address the first two hypotheses.

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291C **PQN-59: a link between cell division and stress granule formation?** Adriano Pizzella, Simona Abbatemarco, Monica GottaPHYM, Universite de Geneve

When exposed to stressful conditions, eukaryotic cells respond by inducing the formation of stress granules (SG), membraneless organelles formed by the condensation of proteins and RNA molecules into liquid droplets.

Our laboratory investigates the function of the ubiquitous PQN-59 (Prion-like-(Q/N-rich)-domain-bearing protein) protein, orthologue of the human SG nucleator UBAP2L and SG component UBAP2. PQN-59 localizes to the SG in embryos and germlines after treatment with various stressors such as heat shock and oxidative stress. Moreover, PQN-59 contributes to proper stress granules formation, as it physically interacts and recruits GTBP-1, a conserved SG component, in this phase separated organelle.

However, PQN-59 depletion also results in brood size reduction, in high embryonal lethality and in chromosome segregation impairments. These unique features make PQN-59 a key player for *C. elegans*' fitness, in a yet unknown fashion.

Our cell biology data and our structure-function analysis of PQN-59 indicate that the role in stress granule formation and the role in embryonic viability and chromosome segregation are independent. To understand the molecular function of PQN-59 outside

stress granule formation, we have performed proteomic analyses in in different genetic backgrounds. Altogether we believe PQN-59 might play fundamental roles in cell homeostasis that have not been described yet.

292C **UNC-10/SYD-2 complex is sufficient to link kinesin-3 to RAB-3 containing synaptic vesicles in the absence of the motor's PH domain** Odvogmed BayansanLife science, Molecular and Cellular Biology, NTHU

Kinesin-3 KIF1A (UNC-104 in *C. elegans*) is the major fast axonal transporter of STVs (synaptic vesicle protein transport vesicles) containing synaptic precursors such as RAB3A (RAB-3) or VAMP2 (SNB-1). Heritable mutations in neuronal motor proteins (and their adaptors) lead to numerous neurodegenerative diseases. The C-terminal PH (pleckstrin homolog) domain of kinesin-3 UNC-104 binds directly to phosphatidylinositol 4,5-bisphosphates that form the lipid bilayers of STVs. We hypothesize that RAB-3-bound STVs employ a dual linker UNC-10/SYD-2 (RIMS1/liprin-alpha in mammals) acting as an UNC-104 receptor. This tripartite RAB-
3/UNC-10/SYD-2 complex would then act as an additional linker to strengthen the motor-lipid interaction. RT-PCR and Western blot experiments reveal a genetic relation between SYD-2, UNC-10 and RAB-3. Co-immunoprecipitation assays demonstrate changes in binding affinities between SYD-2 and UNC-104 depending on the presence or absence of UNC-10 and RAB-3. Bimolecular fluorescence complementation (BiFC) assays exhibit in situ interaction changes between SYD-2 and UNC-104 in either *unc-10* or *rab-3* mutants. In these mutants, UNC-104 travels significantly shorter distances with significantly reduced speeds as compared to wildtype animals. Though both SNB-1 and RAB-3 containing vesicles are actively transported by UNC-104, the movement of RAB-3 containing vesicles is generally enhanced and largely depends on the presence of the dual UNC-10/SYD-2 linker. Strikingly, deleting UNC-104's PH domain did not affect UNC-104/RAB-3 colocalization but did affect UNC-104/SNB-1 colocalization. Further, in worms carrying a point mutation in the PH domain of UNC-104, motility of RAB-3-tagged vesicles remains unaffected while SNB-1-tagged vesicles move with significantly reduced speeds and travel distances in anterograde directions. These findings support the model of a dual UNC-10/SYD-2 linker acting as an additional buttress to connect UNC-104 motor to RAB-3-containing STVs leading to a more stabilized and enhanced transport in neurons.

293C **The enigmatic *trans* function of the Adhesion GPCR Latrophilin cross-talks with the Notch pathway** Willem Berend Post[1], Daniel Matúš[2], Victoria Elisabeth Groß[1], Franziska Fiedler[3], Alexander Knierim[4,5], Torsten Schöneberg[3], Simone Prömel[1,31]Institute of Cell Biology, Heinrich Heine University, [2]The Department of Molecular and Cellular Physiology, The Department of Neurosurgery, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, [3]Rudolf Schönheimer Institute of Biochemistry, Leipzig University, [4]Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig, Leipzig University, [5]Leipzig University Medical Center, IFB Adiposity Diseases

Caenorhabditis elegans provides an excellent model organism to study G protein-coupled receptors (GPCRs) signaling and function in their *in vivo* context to gain insights into how different signals control distinct physiological processes. Thereby, GPCRs are essential cell surface receptors with diverse functions and a huge pharmacological potential. One class of these cell surface receptors are Adhesion GPCRs (aGPCRs). Recently, it was found that some Adhesion GPCRs are not only able to mediate classical signals via G proteins, but also function independently of the seven transmembrane (7TM) domain and C terminus. This *trans* (7TM-independent) function is highly unusual for GPCRs and has interesting functional implications.

To investigate this unusual *trans* mode of action only mediated via the extracellular domain we studied the Adhesion GPCR Latrophilin (LAT-1), which can be considered a prototype of the class as they are among the evolutionarily oldest members of the class. We found that the *trans* function of LAT-1 is involved in germ cell proliferation. In the absence of the receptor, worms display an altered amount of proliferative germ cells. This effect can be rescued to wild-type levels with the sole N terminus of the receptor. Together with the fact that LAT1 acts non-cell autonomously from the somatic gonad (in particular the distal tip cell) to mediate this effect, this demonstrates that the receptor realizes its function via the *trans* mode.

One major pathway controlling cell proliferation and differentiation in the germline is the Notch pathway. Functional and expression analyses showed that LAT-1 is interfering with this pathway. A Notch activation assay revealed that in the absence of LAT-1, the Notch receptor GLP-1 shows lower activation than when LAT-1 is present, suggesting that the aGPCR has an positive modulatory effect on Notch signaling. Further, Bimolecular Fluorescence Complementation (BiFC) analyses lead to the hypothesis that LAT-1 can physically interact with the Notch ligand LAG-2.

In summary, we have shown that the *trans* function of the Latrophilin homolog LAT-1 modulates germ cell proliferation by cross-talking with the Notch pathway in *C. elegans*. This implicates that aGPCRs can be novel potential regulators of this highly conserved signaling cascade.

294C **Kinetics of nucleolar droplets during nucleolar assembly** Sara Zdanovskis[1], Stephanie C. Weber[21]Biology, McGill University, [2]McGill University

The nucleolus is a biomolecular condensate; it is a membraneless organelle that assembles through liquid liquid phase separation. The *C. elegans* embryo is a useful model to study the kinetics of nucleolar assembly given the rapid and measurable assembly of nucleoli during several cell cycles. In two and four-cell stage embryos, nucleolar proteins (e.g., FIB-1, DAO-5) are dispersed in the nucleoplasm throughout the cell cycle. At the eight-cell stage, they concentrate into many small foci, referred to as extranucleolar droplets (ENDs). Then, either ENDs fuse to the two droplets coarsening at the two sites of active rRNA transcription or soluble proteins leave the ENDs to then recondense at these two droplets. These preferentially coarsening droplets are the two nucleoli. Hence, nucleoli coarsen at the expense of ENDs. We performed a targeted RNAi screen to identify genes, including many involved in pre-rRNA transcription, whose knockdown perturbed nucleolar assembly. Unexpectedly, the depletion of SUN-1/ZYG-12 - a complex embedded in the nuclear envelope known for attaching centrosomes to the nucleus - results in nucleolar droplets of variable number, stability, and size. Indeed, nucleolar proteins condense into either zero, two, three or a dozen stable droplets that do not dissolve or fuse like ENDs; and the size of these droplets ranges from small to abnormally large. Notably, the abnormally large and numerous stable droplets are a phenotype more severe than any other known nucleolar phenotype. Therefore, we use the SUN-1/ZYG-12 knockdown as a tool to perturb droplet coarsening kinetics to identify the biophysical processes underlying nucleolar assembly and phase behavior.

295C **Uncovering moonlighting functions of nucleoporins across species** Paula Monterrubio Asensio[1], Cristina Ayuso García[1], Rafael Rodríguez Daga[2], Peter Askjaer[11]Gene Regulation and Morphogenesis, Centro Andaluz de Biología del Desarrollo, [2]Cell Biology and Biotechnology, Centro Andaluz de Biología del Desarrollo

Nuclear pore complexes (NPCs) are composed of multiple copies of 30-35 nucleoporins (Nups; NPPs in *C. elegans*) embedded in the nuclear envelope (NE) 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 both in human 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. The general hypothesis of this project is 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). 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 certain Nups detach rapidly from NPCs upon exposure to heat stress whereas other Nups remain stable. Unexpectedly, the behaviour of individual Nups seems not to correlate with their position within the NPC. Benefitting from the many genome tagging and mutagenesis efforts in the community, we expect to perform a systematic analysis of most NPC components and thereby obtain a detailed view of Nups in stress resistance.

296C **Cell biology and biochemistry approaches to identify the proteins regulating sperm derived mitochondria degradation** Justine Cailloce[1], Fanny Husson[1], Batool Ossareh-Nazari[2], Lionel Pintard[2], Jorge Merlet[1], Vincent Galy[11]Sorbonne University - CNRS, Institut de Biologie Paris Seine, IBPS, Developmental Biology Laboratory, UMR7622, [2]University Paris Cité - CNRS, Institut Jacques Monod, IJM, UMR7592

Mitochondria are essential organelles in eukaryotic cells; they provide cell energy and are also needed for many cellular pathways as apoptosis. To ensure these different functions they have their own genome, the mitochondrial genome (mtDNA). The genes carried by the mtDNA represent only a small fraction of a cells’ genes but are nevertheless vital. During sexual reproduction and unlike the nuclear genome which comes equally from both parents, the mitochondrial genome is only transmitted from the mother. As a matter of fact, uniparental maternal mitochondrial heredity is the most frequent form of mtDNA transmission found in the animal kingdom and also found in the nematode *C. elegans*. Paradoxically, like in many species, the *C. elegans*’ sperm mitochondria enter the oocyte during fertilization. In the last decade we discovered that despite an important dilution (1:800) of sperm-derived mitochondria compare to the oocyte-derived mitochondria, they are actively degraded by a specific mechanism of mitophagy named allophagy. This implies a very selective recognition mechanism. Despite recent significant progress revealing some factors involved in the degradation processes, to date, the signals carried by the sperm mitochondria which trigger their specific recognition and degradation in the embryo are still unknown. To identify such mark(s), we used complementary cell biology and biochemistry approaches in *C. elegans*. We revisited the suggested role of poly-ubiquitylation in sperm mitochondria degradation with an antibody free approach, using fluorescent TUBES (Tandem-repeated Ubiquitin-Binding Entities), which are peptides with a high affinity for poly-ubiquitin chains and we will present our results. In order to go beyond the classical candidates-based approaches used so far in the field and identify the proteins acting in sperm-derived mitochondria degradation we used a unprecedented and unbiased biochemical approach. To this end, we developed a culture method for large populations of worms as an alternative of liquid cultures that allowed us to establish the allophagy interactome by an indi-

rect proximity labelling method. We will present this work that led to the identification of new factors of the allophagic pathway and our exploration of their function(s) in uniparental maternal mitochondrial heredity.

297C **Cleavage furrow-directed cortical flows bias PAR polarization pathways to link cell polarity to cell division** KangBo Ng[1,2], Nisha Hirani[1], Tom Bland[1,2], Joana Borrego-Pinto[1], Nathan Goehring[1,21]Francis Crick Institute, [2]Institute for the Physics of Living Systems, University College London

During development, the conserved PAR polarity network is continuously redeployed, requiring that it adapts to changing cellular contexts and environmental cues. For example, in the early C. elegans embryo, polarity shifts from being a cell autonomous process in the zygote to one that must be coordinated between neighbors as the embryo becomes multicellular. We sought to explore how the PAR network adapts to this shift in the highly tractable C. elegans germline P lineage. We find that although P lineage blastomeres exhibit a distinct pattern of polarity emergence with respect to the zygote, the underlying mechanochemical processes that drive polarity are largely conserved. Instead, it is the changing nature of the symmetry breaking cue which helps coordinate the geometry of PAR polarity to neighboring cells in the P lineage. Specifically, we show that furrow-directed cortical flows associated with cytokinesis of the zygote induce symmetry breaking in the germline blastomere P1 by transporting PAR-3 into the nascent cell contact as it forms, where it is then positioned to bias downstream PAR polarization pathways. Thus advection of polarity proteins by furrow-directed cortical flows directly links cell polarity to cell division, which could be a general strategy for cells to ensure proper organization of cell polarity within dynamically growing systems.

298C **The Kinetochore microtubule coupler, Ndc80 complex, is repurposed for Dendrite Branching** Henrique Alves Domingos[1], Mattie Green[1], Perry Sanders[2], Dhanya Cheerambathur[11]Institute of Cell Biology, University of Edinburgh, [2]University of Edinburgh

Neurons are polarized cells that contain dendrites, which receive information from neighbouring cells, and axons, which are responsible for information output. Many neuronal types form highly complex arborized dendrites. These tree-like structures are important to determine the synaptic input field and connectome of a neuron. Defects in dendritic arbor formation are correlated with neurodevelopmental diseases. Dendrite branching is driven by remodelling of both actin and microtubule cytoskeleton. Recent work identified a post-mitotic role for kinetochore, the multiprotein structure that links DNA to spindle microtubules during cell division, in dendritic extension, branching and regeneration. The core microtubule binding & signalling activity of the kinetochore resides in the KMN network (Knl1, Mis12, Ndc80 complexes). Work from our lab showed that KNL1 promotes dendrite branching of the mechanosensory neuron, PVD, by modulating actin dynamics. However, it is still unclear whether the microtubule coupling activity of the KMN network is important for dendrite branching. The Ndc80 complex, a tetramer composed of NDC80, NUF2, SPC24 and SPC25 subunits, is the central microtubule binding component of the KMN network. Microtubule binding occurs through the calponin homology (CH) domains of NDC80 and NUF2 as well as the N-terminal unstructured region of NDC80. Here we show that the Ndc80 complex is important for dendrite branching of the neuron PVD. The highly branch dendritic tree of PVD is composed of repeated non overlapping units called menorahs. Using an auxin-based degron system we have shown that degradation of the microtubule binding units of the Ndc80 complex, NUF2 and NDC80, result in fusion defects between menorahs, an increase in ectopic branches and deformed cell body. Moreover, we engineered specific mutations on the Ndc80 complex that are known to disrupt the microtubule binding activity in vitro and are important for chromosome-microtubule interactions in mitosis. These mutants recapitulate the degron phenotypes suggesting that the microtubule binding interface is important for proper dendrite branching. Currently, we are investigating how the Ndc80 microtubule binding function affects dendrite branching. To do this, we are testing whether the actin dynamics are perturbed similarly to KNL1 degradation and identifying neuron-specific interactors of the Ndc80 complex using neuron-specific TurboID based proximity labelling proteomic approaches. Overall, these results show a non-canonical role for the core microtubule binding component at the kinetochore, the Ndc80 complex in dendrite branching.

299C **Using MAPH-9 in *C. elegans* to investigate the formation and maintenance of doublet microtubules on centrioles and in cilia** Nabor Vazquez Martinez, Michael Tran, Jeremy MagescasBiology, Stanford

Cilia are microtubule-based organelles that are important for signaling and/or motility in eukaryotes, and ciliary defects are associated with a range of human disorders known as ciliopathies. A repurposed centriole, basal body, docks at the membrane of a cell to template the formation of a cilium by direct extension of centriolar microtubules. The cytoskeletal core, axoneme, of each cilium is composed of a radially symmetrical configuration of nine doublet microtubules. Unlike microtubules in the cytoplasm made of a single cylinder, doublet microtubules are formed from an incomplete microtubule built off the wall of an existing microtubule. Despite the conservation of this structure across the tree of life, how doublet microtubules form and are maintained *in vivo* is still poorly understood. *C. elegans* provide a unique system in with to probe the mechanisms by which doublet microtubules are assembled and maintained. Unlike most other ciliated organisms, *C. elegans* centrioles have nine singlets

during early development but build microtubule doublets de novo post-mitotically just prior to ciliogenesis. We have identified MAPH-9/MAP9 as a microtubule associated protein that exclusively recognizes doublet microtubules in the cilia of *C. elegans*: MAPH-9 is only expressed in ciliated cells; MAPH-9 localization appears concomitant to the post-mitotic appearance of doublet microtubules; and MAPH-9 localizes to the 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 doublet microtubule defects, dysregulated axonemal motor velocity, and perturbed cilia function. Future studies will use the remarkable specificity of MAPH-9 localization as a handle to better understand how doublet microtubules are formed.

300C **Dynein orients the mitotic spindle during asymmetric seam cell division to ensure proper cell fate and integrity of the epidermis** Cátia Carvalho[1,2,3], Daniel J Barbosa[4,5], Reto Gassmann[3,41]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, [2]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, [3]IBMC - Instituto de Biologia Molecular e Celular, [4]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, [5]Toxrun - Toxicology Research Unit, University Institute of Health Sciences, CESPU

Cytoplasmic dynein 1 (dynein) is a microtubule minus-end-directed motor whose critical functions during cell division include the separation of centrosomes during prophase and the subsequent positioning and orientation of the mitotic spindle. In *C. elegans,* these functions of dynein have been extensively studied in the early embryo but remain poorly explored in other developmental contexts. Here, we use a hypomorphic mutant of dynein intermediate chain (*dyci-1*) to investigate dynein's role during postembryonic development of the epidermis. Epidermal development involves several rounds of stem cell-like divisions, in which unequal distribution of Wnt pathway components results in the differentiation and fusion of anterior seam cell daughters with the hyp7 syncytium, while posterior daughters retain seam cell identity. Epidermal development concludes at the L4 stage, when the longitudinally arranged row of seam cells forms a syncytium. Live imaging of the last round of seam cell divisions shows that *dyci-1* mutant cells separate centrosomes normally but frequently fail to align the centrosome-centrosome axis with the anterior-posterior axis of the seam cell row. This results in mis-orientation of the mitotic spindle, and, consequently, seam cell daughters become incorrectly positioned outside of the seam cell row. In mis-oriented divisions, the Wnt pathway component APR-1 is no longer asymmetrically distributed to daughter cells, and, consistent with altered cell fate, mutant L4 animals have fewer terminally differentiated seam cell nuclei and contain cells that have lost seam cell identity but have failed to fuse with the hyp7 syncytium. Mutant L4 animals have prominent gaps and discontinuities in the seam cell syncytium, which correlates with a permeable cuticle and a dramatically reduced life span due to spontaneous rupture shortly after reaching adulthood. We conclude that by orienting the centrosome-centrosome axis along the anterior-posterior axis during prophase, dynein ensures proper seam cell fate during asymmetric divisions and the integrity of the epidermal tissue.

301C **Nonlinear antagonism and control of oligomerisation in the PAR polarity network in the *C. elegans* embryo** Alex Chizh, Nathan GoehringFrancis Crick Institute

The anterior-posterior axis of the *C. elegans* embryo is defined at the one-cell stage by asymmetric cortical enrichment of proteins constituting the highly conserved PAR network. The anterior and posterior PAR proteins promote their own membrane association, while antagonising opposing proteins. Stable membrane patterning requires nonlinear interactions between proteins, such as switch-like or ultrasensitive responses to dosage, the sources of which are not well understood in the PAR network.

PAR-3, an oligomerising scaffold protein and hub, is abundant in interconnections and feedback between PAR protein reaction kinetics, protein motility and spatial organisation. These features, in PAR-3 and other proteins, appear to be important targets of regulation in the PAR network. In combination with the reaction kinetics of PAR antagonism, they can contribute to generating switch-like responses and, consequently, stable pattern formation. PAR-3 forms a highly heterogeneous and dynamically regulated population of clusters on the membrane, differing in both size and motion. By investigating the control of PAR-3 assembly, we aim to characterise how non-equilibrium and statistical physical processes can contribute to intracellular pattern formation.

302C **Endogenous tagging of the *C. elegans* GABARAP ortholog LGG-1 reveals a highly dynamic microtubule-associated tubular network of LGG-1 required for autophagy** Julius Adam, Ludovico Alves, Stefan EimerDepartment of Structural Cell Biology, Institute for Cell Biology and Neuroscience, Goethe University Frankfurt

The Atg8/LC3/GABARAP family of ubiquitin-like proteins have been shown to be required for the formation and maturation of the autophagosome during autophagy. GABARAPs have been initially described as microtubule (MT)-associated proteins, however, so far this MT association has not been shown in live cell imaging. When ectopically expressed in mammalian cells or *C. elegans*, GFP-tagged Atg8 proteins and their orthologues form discrete puncta in the cytosol, thought to resemble sites of active autophagy. In contrast, by fluorescently tagging of the endogenous GABARAP ortholog LGG-1 in *C. elegans* using CRISPR/Cas9 genome editing, we show a highly dynamic network of mCherry-LGG-1 tubules in addition to the previously described discrete

puncta. We could demonstrate that the formation of this network depends on the N-terminal α1/α2 helix of LGG1. By coexpression of the endogenous tagged LGG-1 and the microtubule associated protein MAPH1.1, we confirmed a high level of association of the LGG-1 network with microtubules using spinning disc confocal live cell imaging. In addition, by depleting ATP using sodium azide, we could also show that LGG-1 network dynamics are dependent on ATP. In further experiments, we discovered LGG-1's network-structure to be required for autophagy-dependent phenotypes like insulin signaling pathway related longevity and overcoming external stressors such as lysosomotropic drugs.

In contrast to previously tagged GFPLGG1, the endogenous nature of the LGG1 reporter allows for live cell imaging of autophagy-related processes in *C. elegans* without overexpression of LGG1. Therefore, it is reducing the potential of artificially created phenotypes. Most importantly, it led to the discovery of LGG-1's network-structure while still displaying previously described discrete puncta.

303C **Characterization of gonad morphology in a low-fertility mutant with defects in dynein and MEL-28** Julia Stobierska, Gabriela Vida, Anita FernandezBiology, Fairfield University

We have been studying how dynein, a minus-end directed microtubule motor, and MEL-28, a protein required for rebuilding the nuclear pore after mitosis, interact to affect fertility in *C. elegans.* The *or283ts* mutant allele of *dhc-1*, the gene that encodes the large subunit of dynein, has minimal impact on fertility. *mel-28(t1684)* single mutants also produce a normal brood size. In contrast, the *dhc-1; mel-28* double mutant has a severely reduced brood size. This suggests that dynein and MEL-28 act in parallel to promote fertility in *C. elegans*. An egg-laying experiment showed that initially, young adult *dhc-1; mel-28* double mutants lay eggs at the same rate as each single mutant. However about eight hours after reaching adulthood, the egg-lay rate of *dhc-1; mel-28* double mutants dramatically slows. To determine whether this low fecundity is associated with aberrant gonad morphology, we generated mutants that express a cell membrane component fused to mCherry and a GFP-tagged chromatin marker. We used fluorescence microscopy and these markers to characterize phenotypes in wild-type animals, *dhc-1* single mutants, *mel-28* single mutants, and the *dhc-1; mel-28* double mutants. Each single mutant looked very similar to the wild type. In the double mutants, the proximal gonads show a variety of mutant phenotypes, including small and rounded oocytes, lack of chromatin, and conglomerated chromosomes. In the distal gonad, double mutants have rounded and multinucleated compartments and an occluded lumen. Despite these differences, the length of the gonads, from the distal tip to the -1 oocyte, is not significantly different between the wild type and the mutants. The mutant phenotypes we observed show that double disruption of dynein and MEL-28 interfere with the proper formation of the oocytes, which could explain the low-fecundity phenotype.

304C **Reduced Brood Size in *dhc-1; mel-28* Double Mutants is Associated with Defective Yolk Import** Anna Weissenberg, Anita FernandezBiology, Fairfield University

Dynein is a multi-protein molecular motor that ferries cargo toward the minus end of microtubules. MEL-28 is a protein with roles in the nuclear pore and in chromosome segregation. We have been studying genetic interactions involving *dhc-1*, which encodes the largest subunit of dynein, and the *mel-28* gene. Compared to the wild type and to single mutants, *dhc-1; mel-28* double mutants have significantly reduced brood sizes. Coelomocyte uptake and yolk import are intercellular trafficking events that require microtubule motor activity, so we decided to test these to determine if either process is affected in the double mutants. In order to test the activity of coelomocytes during endocytosis, we crossed a myo3::ssGFP transgene to the double mutants and to both single mutants. Animals with this transgene express GFP from muscle cells that are released into the pseudocoelom, or body cavity. In normal animals this secreted GFP is then endocytosed by the coelomocytes, which are scavenger cells that remove debris from the pseudocoelom. We measured the ratio of GFP fluorescence in coelomocytes versus the pseudocoelom and found that there was no significant difference in the coelomocytes' endocytosis ability amongst the strains we tested suggesting that the reduced overall brood size in *dhc-1; mel-28* double mutants is unrelated to the activity of endocytosis by coelomocytes. We also crossed a YP170::tdimer2 transgene to the mutants to study the effect of each genetic lesion on endocytic trafficking of yolk protein from the intestine to the C. elegans oocytes. Compared to the wild type and both single mutants, the *dhc-1; mel-28* double mutants have a significantly reduced ability to take up yolk protein into the maturing oocytes. Instead, the tagged yolk protein accumulates in the surrounding pseudocoelom. This suggests that the low brood size in *dhc-1; mel-28* mutants could be due to the reduced ability of receptor-mediated endocytosis of the yolk protein into the oocytes.

305C **Defects to Dynactin cause Sperm Function Deficiencies that are Partially Rescued by Defects in Y-Complex Nucleoporins** Sydney Youd, Kaitlin Levangie, Aura Cristina Spar, Anita FernandezDepartment of Biology, Fairfield University

Dynactin is a multi-protein machine that activates the microtubule motors dynein and kinesin. *dnc-1* encodes the p150(Glued) subunit of dynactin. *dnc-1(or404ts)* mutant hermaphrodites produce a low brood size at restrictive temperature. About half the oocytes laid by self-fertilized *dnc-1* mutant hermaphrodites are unfertilized oocytes, suggesting a defect with fertilization.

To determine if *dnc-1* mutants have defective sperm, we mated mutant *dnc-1* males to *fog-2(qC1)* females. These matings produced a low brood size with a low fertilization rate, suggesting that both *dnc-1* mutant hermaphrodites and *dnc-1* mutant males produce inefficient sperm. When wild-type males mate with wild-type hermaphrodites, the male sperm outcompete the hermaphrodite sperm such that most of the progeny are sired by the male. We mated marked *dnc-1* mutant males to wild-type hermaphrodites and found that less than half the progeny were sired by the mutant males. This showed that *dnc-1* mutant male sperm cannot outcompete wild-type hermaphrodite sperm, and it supports the idea that defects to *dnc-1* impact sperm competence.

In the course of studying interactions involving MEL-28, dynein, and its regulators, we generated *mel-28 III; dnc-1 IV* double mutants. *mel-28* encodes a component of the Y complex of the nuclear pore, which is recruited early to chromatin during the re-building of the pore at the end of mitosis. Hermaphrodites homozygous for the *mel-28(t1684)* mutation produce 100% inviable embryos, but have no defects in brood size and do not lay unfertilized oocytes. The *t1684* mutation in *mel-28* partially rescues the brood size, fertilization rate, and sperm competition defects in *dnc-1* mutants. To determine if other Y-complex components play a similar role in sperm, we made an *npp-5(ok1966) II; dnc-1(or404ts) IV* double mutant and studied its fertility. The double mutant hermaphrodites had larger brood sizes and a higher fertilization rate than *dnc-1* single mutants. This supports the idea that defects to Y-complex components rescue sperm problems caused by defective dynactin. *C. elegans* sperm do not have a nuclear envelope, but our data suggest that there is still a function for the Y-complex in sperm. We favor the idea that the Y complex has a non-nuclear-pore function in sperm that acts in opposition to dynactin.

306C **Adaptation of fungal bioluminescence for protein tagging in *C. elegans*** Liam Schuck, Ryan DoonanGlow Worms, The University of Texas at Austin

Fluorescent protein tagging is a powerful research tool that allows for the visualization of protein localization, expression, and function; however, fluorescent imaging requires expensive fluorescent microscopes and causes photobleaching of fluorophores over time, making the fluorescence of the protein disappear. Bioluminescence is an alternative to fluorescence that could alleviate both of these issues. In this study, we have adapted a bioluminescent tag derived from *Neonothopanus nambi* for use in *Caenorhabditis elegans*. This particular tag allows for autonomous bioluminescence without substrate feeding. The tag functions via a reaction between a luciferin (hispidin) and the luciferase protein tag. The biosynthetic pathway for the luciferin was cloned into a mosSCI targeting plasmid and injected into the *C. elegans* gonad for stable transgenic expression. The luciferase was fused to multiple endogenous proteins via Cas9 insertion and the signal was compared to that of the fluorescently-tagged protein. Furthermore, the biosynthetic pathway and the luciferase tag were randomly mutagenized to optimize the exogenous pathway for protein tagging. This system provides a low-cost and user-friendly protein tagging strategy that can be seen with both the naked eye and under simple compound microscopes.

307C **The role of the kinesin, KLP-4, in local protein synthesis** Jasmine Tang, Jessika Linnemeyer, Jay PieczynskiRollins College

The primary, but not exclusive, function of kinesin motor proteins is to traffic vesicles and/or organelles toward the plus ends of microtubules. Both *ex vivo* cell-based approaches and *in vitro* assays have repeatedly and thoroughly characterized the biochemical properties of kinesins, including monomer to dimer transitions, intramolecular autoinhibition, and engagement with microtubules via their motor domains. However, identification of the specific cargoes for specific kinesins have not been fully addressed. To this end, we have taken a combined genetic and proteomic approach to identifying potential cargoes for the worm kinesin, KLP-4. To identify potential interacting proteins and cargoes, we performed large-scale immunoprecipitations on mixed stage animals using an endogenously 3X-Flag-tagged version of KLP-4. Following these pulldowns, we performed LC-MS/MS to identify potential interacting peptides. From these assays, we identified 331 novel proteins, 32.6% of which are involved in protein production, including numerous ribosomal associated proteins. To compliment these pulldowns, we also performed a similar experiment, this time UV-crosslinking followed by proteinase digestion. Using this methodology, we also found a significant RNA component to the KLP-4 interactome. These results suggest that KLP-4 plays a role in local protein synthesis, trafficking ribosomes and potentially RNAs to sites for “on demand” translation.

308C **Ciliary distribution and ectocytosis of the tetraspanin TSP-6** Adria Razzauti Sanfeliu[1], Christine Bruggeman[2], Guus Haasnoot[2], Teresa Lobo[1], Aleksandra Nawrocka[1], Erwin J.G. Peterman[2], Patrick Laurent[11]Neurophysiology, UNI, ULB Neuroscience Institute, Université Libre de Bruxelles, [2]Department of Physics and Astronomy and Laser Centre, Vrije Universiteit

Sensory cilia dynamically concentrate receptors and associated proteins to organise and tune signal transduction. Yet, how cilia protein homeostasis is maintained remains to be understood. Proper signaling involves the control of receptor entry and exit from the cilia and/or the local endocytosis of receptors at the ciliary base. More recently, the shedding of Extracellular Vesicles (EVs) carrying receptors was suggested to contribute to cilia homeostasis. Using TSP-6 protein -a marker enriched on ciliary EVs- we observed EV biogenesis from most *C. elegans* ciliated neurons. We used time-lapse fluorescence imaging and single-mol-

ecule tracking experiments to elucidate the dynamics underlying the ciliary distribution and the formation of EVs containing TSP-6. In wild type conditions, TSP-6 concentrates at the cilia transition zone and tip compartments. We observe pathological accumulations of TSP-6 in mutants that are involved in ciliary entry, traffic, exit and endocytosis. These TSP-6 accumulations can occur all along the cilia and correlate with local ciliary EVs biogenesis. The capture of positive ciliary EVs by cilia-supporting glia is observed and used as a proxy to quantify the production of EVs from the cilium. Altogether our work shines a light on the ciliary EV biogenesis process.

309C **A novel role of kinesin-1 in localised protein synthesis?** Astrid Boström, Gino Poulin, Viki AllanFaculty of Biology, Medicine, and Health, University of Manchester

Cargo transport by motor proteins is essential in elongated, polarised cells such as neurons in order to replenish synaptic proteins and remove waste products. While the transport of membranous organelles and vesicles is well-studied, how motor proteins transport cytoplasmic proteins is more elusive. Furthermore, spatial constraints in cells with complicated morphologies make them reliant on localised protein synthesis in order to maintain the local protein supply. In *Caenorhabditis elegans* and mammalian cells, ribosomes and mRNA have been found both in dendrites and at the axon tip. However, the transport and localisation of the multiaminoacyl-tRNA synthetase complex (MSC), a fundamental part of the protein synthesis machinery, has not been previously explored. Kinesin-1 is an anterograde microtubule motor with two identical heavy chains and two identical light chains. A proximity labelling mass spectrometry approach (bio-ID), aimed at detecting novel cargoes specific to distinct kinesin-1 light chains, identified all nine components of the MSC as near-neighbours of KLC2 in HeLaM cells. In this study, co-immunoprecipitation experiments in mammalian cell lines support this observation, suggesting that MSC components are interactors of kinesin-1 with a preference for KLC2 over KLC1B. Furthermore, we generated *C. elegans* strains where fluorescently tagged MSC components are expressed downstream of pan-neuronal or neuron-specific promoters. Using spinning disc live microscopy, we observed trafficking of MSC components in both neuron cell bodies and neurites. We propose that the complex is transported along neurons by motor proteins including kinesin-1 in order to supply the local protein synthesis machinery and maintain synaptic plasticity.

310C **Galectin is required for apoptotic cell removal in *Caenorhabditis elegans*** Yu-Shin Chang, Yu-Chun Chao, Ting-You Lee, Che-Wei Chang, Yi-Chun WuInstitute of Molecular and Cellular Biology, National Taiwan University

Apoptosis is a crucial process for the development and homeostasis of metazoans, and proper clearance of apoptotic cells is essential for tissue homeostasis. Dysregulation of this process has been implicated in cancer and autoimmune diseases. Despite its importance, the underlying molecular mechanisms are not fully understood. Here, we discovered a novel function for a member of the galectin family, which recognizes β-galactosides, in the degradation of apoptotic cells in *C. elegans*. Using genetic depletion of the galectin, we observed accumulation of apoptotic cells in the adult germline. Time-lapse recordings revealed that mutants lacking the galectin showed a defect in lysosomal recruitment and incorporation of lysosomal enzymes into late phagosomes, while the recognition and engulfment processes appeared normal. Consistently, acidification of apoptotic cell corpses during cell corpse removal was defective. These results suggest that the galectin is involved in the late stages of phagosome maturation, specifically in the fusion of lysosomes with late phagosomes for efficient degradation of apoptotic cells. Intriguingly, we also observed that the galectin was localized to pseudopods during their extension around apoptotic cells as well as late phagosomal and phagolysosomal surface. Therefore, the galectin may have a redundant or dispensable role in engulfment. We are currently investigating the molecular mechanism underlying the galectin's role in lysosome recruitment and fusion. Specifically, we are working to identify the specific galectin-interacting partners during these processes and to characterize the structural and biochemical features of the galectin that are critical for its function in this context.

311C ***C. elegans* Meiotic and Mitotic Function is Regulated by a Mitochondrial Localized Protein** Samantha H Schaffner[1], Maulik R Patel[1,21]Biological Sciences, Vanderbilt University, [2]Cell and Developmental Biology, Vanderbilt University

Mitochondria play many essential cell biology roles, such as in apoptosis and immune regulation, that go beyond their canonical function of energy production. A nuclear-encoded mitochondrial-localized *Caenorhabditis elegans* protein that could be playing a non-canonical role in mitochondrial function is SPD-3. This protein is essential for the fertility of the animal as *spd-3* mutants have a sterile phenotype due to disruption of meiotic and mitotic spindle regulation which results in abnormal polar body extrusion and chromosome segregation. Despite its function in the nucleus, previous research has shown that ectopically introduced SPD-3::GFP localizes to the mitochondria. While it might be assumed that SPD-3 has some function in mitochondrial ATP production, such specialized effects on fertility are not typically seen in nuclear-encoded elements of cellular respiration function. Therefore, we hypothesize that *spd-3* is a mitochondrial-localized regulator of meiosis and mitosis that must have a specific and novel mitochondrial function. Our research aims to show the sub-organellar localization of SPD-3 at the endogenous locus to more clearly characterize how this protein might signal outside of the mitochondria. Additionally, we will show the results of a genetic screen being conducted to identify potential genetic interactors of *spd-3* and therefore possible signaling

pathways that involve *spd-3*.

312V **Specificity in glia-neuron interactions** Sneha Ray, Pralaksha Gurung, Richard S Manning, Alexandra Kravchuk, Aakanksha SinghviFred Hutch Cancer Research Center

Nervous systems consist of two cell-types, neurons and glia. Glia interact with neurons physically and molecularly to modulate neuron shape, functions and animal behavior. One site of this interaction is the neuron receptive-ending (NRE), where a neuron receives input from either the outside world or other neurons. While each glia can associate with multiple NREs, it is unclear whether its cell:cell interactions are similar or different between each pair. It is known, however, that a single glia responds differently to distinct NRE activities, suggesting that glia-NRE cell-cell interactions are non-uniform. Determining this logic of glia-neuron interaction specificity is critical to understand how glia impact nervous system funciton.

To examine this at single-cell resolution, we focused on a single glia in *C. elegans*, the amphid sheath (AMsh) glia, which invariantly contacts NREs of 12 distinct sensory neurons. We previously found that the AMsh glial cation-chloride transporter KCC-3 regulates shape, function, and associated animal behavior of the NRE of the AFD thermosensory neuron.

AMsh glia have apical-basal polarity, and we find that KCC-3 localizes to a microdomain within apical membranes only at AFD-NRE contact site and not around other NREs. To ask how it achieves this micro-polarity domain, we performed structure-function, genetic, and cell-ablation studies. This uncovered a two-step process. First, a 55 amino-acid intracellular N-terminal sequence determines KCC-3 apical-basolateral membrane targeting in the polarized glial cell. Then, C-terminal sequences guide apical microdomain restriction to AFD NREs. Surprisingly, this refinement is not driven by recruitment of KCC-3 by AFD-NRE, but instead repulsion from other ciliated NREs (minimally AWC-NRE + 1 other NRE). Functionally, we find that KCC-3 apical microdomain localization is required for AFD-NRE shape regulation. Unexpectedly, *kcc-3* loss also impacts behaviors regulated by non-AFD NREs. Thus, glial KCC-3 can modulate cross-modal sensory neuron functions. We are currently assessing whether mis-localized KCC-3 also affects non-AFD NRE shape and function.

Together, our studies reveal that a single polar glial cell can maintain molecular microdomains around contact-sites of specific NREs. Our data also reveal mechanistic insight on how this is accomplished, and suggests that this cellular specificity in glia-neuron interaction may broadly impact nervous system functions.

313V **Formation, growth and function of the axonal spectrin lattice** Grace Swaim, Oliver V Glomb, Shaul YogevNeuroscience, Yale School of Medicine

A conserved periodic lattice of actin rings and spectrin tetramers protects axons from breakage and degeneration. How the Membrane Periodic Skeleton (MPS) forms and what it functions are remains poorly understood. Using STED and SIM microscopy in combination with newly developed conditional labelling approaches, we were able to visualize the axonal transport of endogenous spectrin and its incorporation into the MPS lattice at single axon resolution in *C. elegans.* Surprisingly, spectrin transport is bimodal, comprising fast intermittent runs observed for other cargo but also movements that are 100-fold slower than previously reported. We identified the conserved coiled-coil proteins UNC-76/FEZ1 and SCOC/UNC-69 as spectrin- kinesin-1 adaptors that mediate both ultra-slow and stop-and-go movements. Loss of either protein disrupted spectrin transport, prevented distal MPS formation and resulted in distal axon degeneration. Following transport, we found that newly synthesized spectrin integrates into the MPS lattice at discrete hotspots, which grow in size to drive the scaled expansion of the MPS at the rate of axon elongation. Last, single cell degradation of spectrin or its transport adaptors led to drastic cytoskeletal rearrangements, including excessive microtubule movements and misdistribution. These movement were caused by ectopic foci of actomyosin at microtubule tips, suggesting that one of the functions of the MPS is to restrict actomyosin contractility to the axonal membrane. Together, our results provide new mechanistic insights into the formation, expansion, and function of the membrane periodic skeleton.

314V **Exophergenesis response to expression of aberrant intrinsically disordered proteins** Edward Chuang, Monica DriscollMolecular Biology and Biochemistry, Rutgers University

Neurodegenerative diseases are devastating afflictions that affect millions of Americans. Protein aggregation is a hallmark of pathology across most neurodegenerative diseases, although biophysical properties and toxicity features of aggregates remain poorly understood. Aggregation pathology spreads through the brain along neuronal circuits, suggesting a mechanism of neuronal transfer of aggregates that may be targeted for novel interventions against these devastating neurodegenerative diseases. How the biophysical properties of aggregates relate to their transfer remains a critical question. Our lab recently observed that *C. elegans* neurons can expel aggregated mCherry and PolyQ protein from the soma in large membrane-bound vesicles (called exophers) to promote proteostasis within the neuron. However, it is not known how other potential cargoes are handled by the exopher apparatus. We focused on FUS and TDP43, two RNA-binding proteins with intrinsically disordered prion-like domains that are commonly found to aggregate in the neurons of patients with Amyotrophic lateral sclerosis. Intrinsically disordered

proteins such as FUS and TDP43 can form various material states including liquid droplets, hydrogels, and insoluble aggregates. The biophysical state of FUS and TDP43 protein can be altered by disease-linked mutations, often resulting in less soluble forms. In a second approach, we have found that neuronal exophers can contain foci of transgenically introduced PGL-3, an intrinsically disordered P-granule protein known to form liquid droplets. It is unknown whether altering the phase behavior of cargo protein can alter exopher biology. We, therefore, expressed disease mutant forms of FUS and TDP43 in *C. elegans* touch neurons and engineered PGL-3 derivatives with varied phase behavior to determine their recruitment to exophers and their effects on exophergenesis. The results presented here further our understanding of exopher biology and the potential for exophers to be a target for neurodegenerative disease intervention.

315V **EleganSeg: Whole body instance segmentation using improved U-Net in *Caenorhabditis elegans* microscopic images** Pablo-Emmanuel Layana-Castro[1], Antonio García-Garví[2], Konstantinos Kounakis[3,4], Joan Carles Puichalt[2], Ilias Gkikas[4,5], Ioannis Tsiamantas[4,5], Nektarios Tavernarakis[3,4], Antonio-José Sánchez-Salmerón[21]Instituto de Automática e Informática Industrial, Universitat Politècnica de València, [2]Instituto de Automática e Informática Industrial, Universitat Politécnica de Valencia, [3]Department of Basic Sciences, Faculty of Medicine, University of Crete, [4]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, [5]Department of Biology, School of Sciences and Engineering, University of Crete

Caenorhabditis elegans is a great model for the investigation of organismal, cellular and subcellular biology through optical and fluorescence microscopy, with ever expanding research applications. In order to be able to acquire quality data however, these applications typically require that the researcher spends a lot of time rigorously studying lots of images with large numbers of worms, manually labeling features and regions of interest (ROI) before the analysis can be performed by appropriate software (usually ImageJ). This is a tedious and laborious process that can represent a major chokepoint in an experimental pipeline (with analysis often requiring significantly more time than image acquisition). In addition, it can be quite vulnerable to user error and bias. Thankfully, advances in deep learning technology have created the possibility to streamline the process by utilizing Deep Neural Networks that have been appropriately trained to automatically identify and segment regions of interest from images of worms. Here we present EleganSeg, a whole-body instance segmentation model we have developed based on a U-Net neural network architecture. This model is designed to quickly generate precise masks/ROIs for individual worms in highly populated images and even be able to correctly resolve individuals in cases of significant worm overlap.

316V **Understanding how lipid metabolism contributes to α-synuclein toxicity in *C. elegans*** Tao Zhang[1], Renée Seinstra[1], Jorien van der Weerd[1], Suzanne Couzijn[1], Maria Eugenia Goya[1], Folkert Kuipers[1], Rebecca Heiner-Fokkema[1], Ellen Nollen[21]University Medical Center Groningen, [2]University of Groningen, University Medical Center Groningen

Parkinson's disease is a neurodegenerative disease characterized by abnormal accumulation and aggregation of α-synuclein protein. Though a bidirectional relationship between lipid metabolism and α-synuclein toxicity was proposed, the detailed interplay remains unresolved. To investigate how α-synuclein toxicity affects lipid homeostasis in *Caenorhabditis elegans (C. elegans)*, unbiased lipidomic analysis showed triacylglycerol (TG) was the major lipid that was drastically downregulated in wild-type α-synuclein worm compared with YFP control worm. Oil red O staining confirmed a significant decrease in triacylglycerol level in α-synuclein worms, while α-synuclein knockdown rescued the triacylglycerol level. Combining a targeted RNAi screen and our in-house wide field-of-view nematode tracking platform (WF-NTP), potential modifiers of α-synuclein toxicity were found in both lipogeneses and lipolysis pathways. Based on these results, we hypothesize that α-synuclein causes cellular toxicity by interfering with a specific step in cellular lipid metabolism. Further explorations are needed to elucidate the mechanism by which α-synuclein influences lipid metabolism during the onset and progression of Parkinson's disease.

317V **Exploiting the CRISPR/Cas9 system to label chromosomes of *C. elegans* gonads** CRISTINA PINEIRO LOPEZ[1], Simone Koehler[21]Cell Biology and Biophysics, European Molecular Biology Laboratory, [2]European Molecular Biology Laboratory

One of the most impressive activities of chromosomes during meiosis is their ability to form pairs with their homologous partners. The pairs of homologous chromosomes are then held together by the assembly of the synaptonemal complex and recombination which physically links pairs of homologous chromosomes. Studying the dynamics of chromosomes during meiosis requires tools to visualize not only proteins but also individual pairs of homologous chromosomes. Such techniques should ideally preserve the chromosome morphology and DNA topology to detect changes in chromosome conformation. Traditionally, DNA Fluorescence *In Situ* Hybridization (FISH) has been used to label chromosomes. This technique requires DNA denaturation and uses disruptive chemicals to hybridize the fluorescent DNA probes to a gene or locus of interest. Additionally, this harsh treatment may disrupt the physical interactions between proteins and DNA, or the structure of proteins that are responsible for holding homologous chromosomes together, which impedes accurate co-labelling of DNA and proteins, and may lead to a misinterpretation of the data. Recently, the CRISPR/Cas9 system has been repurposed to label chromosomes. However, it has only been tested in cell culture systems. I will show how the CRISPR/Cas9 system can be applied to label chromosomes in

whole-mounted tissue of *C. elegans* gonads. After several rounds of optimization, I obtained a reliable and quick protocol to label multiple chromosomes simultaneously by using dCas9-Halo protein labelled with different fluorophores. Importantly, this technique allows us to label chromosomes together with proteins and RNA, as the gentle dCas9 based labelling protocol does not interfere with immunostaining or RNA-FISH protocols. My new protocol will thus facilitate studying the behavior of chromosomes throughout the germline and will allow us to shed light on the mechanisms that ensure accurate pairing, synapsis, recombination, and segregation of homologous chromosomes during meiosis.

318V **Mutagenesis and structural modeling implicate RME-8 IWN domains as conformational control points** Anne Norris[1], Collin T McManus[2], Rouchen Ying[2], Simon Wang[2], Barth D Grant[31]MBB, Rutgers University, [2]Rutgers, [3]MBB, Rutgers

After endocytosis, transmembrane cargo is differentially sorted into degradative or recycling pathways. This process is facilitated by recruitment into physically distinct degradative or recycling microdomains on the limiting membrane of individual endosomes. Endosomal sorting complexes required for transport (ESCRT) mark the degradative microdomain, while the recycling domain is marked by the retromer complex and associated proteins RME-8 and SNX-1. The separation of endosomal microdomains is also controlled by RME-8 and SNX-1, at least in part via removal of degradative component HRS/HGRS-1 from the recycling microdomain. This activity is likely due to recruitment and activation of chaperone Hsc70 on the endosome by the RME-8 DNAJ domain. To better understand the mechanism of RME-8 function we performed a new phylogenetic analysis of RME-8 and identified new conserved sequence features. In a complementary approach, we performed structure-function analysis that identified the C-terminus as important for microdomain localization and likely substrate binding, while N-terminal sequences beyond the known single N-terminal PH-like domain are important for endosome recruitment. Random mutagenesis identified IWN4, and by analogy IWN3, to be important for the autoinhibitory DNAJ domain binding, with IWN3 playing a critical role in HRS uncoating activity. Combining AlphaFold structural predictions with *in vivo* mutation analysis of RME-8, we propose a model whereby SNX-1 and the IWN domains control the conformation of RME-8 and hence the productive exposure of the DNAJ domain. Furthermore, we propose that the activation of RME-8 is cyclical, with SNX-1 acting as an activator and a target of RME-8 uncoating activity.

319V **First characterization of the *C. elegans* WASH complex in endocytic recycling** Patricia Irizarry-Barreto, Jennifer Smolyn, Luigy Cordova-Burgos, Martha SotoRutgers - RWJMS

Branched actin networks support epithelial polarity, in part by maintaining polarized protein transport. For example, loss of the branched actin regulating WAVE complex results in apical/basal defects of epithelia, including mislocalization of the Cadherin protein, a major regulator of apical/basal polarity. WAVE and Cadherin colocalize at key transport organelles, including RAB-11-positive recycling endosomes, and at the Golgi. Therefore, understanding how branched actin contributes to polarized events is essential for understanding epithelial polarity. However, in *C. elegans*, only two Nucleation Promoting Factors (NPFs) that regulate branched actin through Arp2/3 have been examined, WAVE and WASP. We therefore analyzed the orthologs of the third *C. elegans* Apr2/3 NPF, the WASH complex. The WASH complex is similar to the WAVE complex, in that both complexes include 5 paralogous components. Surprisingly, only 4 of the 5 components had been identified in *C. elegans*, and the role of WASH in trafficking had not been described. We used existing mutations, RNAi and CRISPR to determine the effects of loss of the WASH complex, focusing on two epithelial tissues: the adult intestine and the embryonic epidermis. We used our phenotypic assays to test a candidate for the missing 5th WASH component, the FAM21 proposed ortholog, CO5G5.2. Loss of any WASH component, using mutations or RNAi, resulted in the same defect: defective transport of cargo on RAB-5 and PI(3)P-positive early endosomes, suggesting *C. elegans* WASH regulates sorting during endocytic recycling, through retrograde trafficking to the Golgi apparatus. Therefore, *C. elegans* has a conserved, 5-member WASH complex, that appears to function, like its orthologs, in early endosome to Golgi retrograde transport. Our analysis further identifies unique features of the *C. elegans* WASH complex that have implications for its regulation.

320V **The non-canonical role of the kinetochore protein KNL-1 in axon formation** Vasileios-Rafail R Ouzounidis, Mattie Green, Rakshitha R Madamakki, Dhanya Cheerambathur Institute of Cell Biology, University of Edinburgh

The nervous system is composed of highly polarized cells, neurons, that form an interconnected network to relay information. Proper transmission of information relies on the two main compartments within the neurons, dendrites that receive information and axons that relay the information to other cells. Axon outgrowth and branching are vital steps to ensure the correct connectivity of the nervous system. Proper axon outgrowth is linked with axon pathfinding and axon branching allows neurons to form unique patterns of connectivity through synaptic contacts. During axon formation, actin and microtubule filaments, undergo dramatic rearrangements to guide, shape and provide structural support to the axon. Although we know much about the cytoskeletal regulation during axon outgrowth, very little is known about the regulation of axon branching.

Here, we describe an unexpected post-mitotic role for the chromosome segregation machinery in axon branching. The highly conserved outer kinetochore protein network is the core microtubule binding component of the kinetochore, the structure

that tethers chromosomes to spindle microtubules, during chromosome segregation. Within the outer kinetochore, the protein KNL-1 serves as a scaffold for the microtubule binding and signaling activity of the kinetochore. Mutations in KNL-1 are linked to neurodevelopmental disorders such as primary microcephaly. We have identified a non-canonical cell division independent role for KNL-1 in axon and synapse formation in the touch receptor neurons.

Using a neuron-specific split-GFP system, we found that KNL-1 is enriched in the developing axons of the touch receptor neurons. Specifically, KNL-1 accumulates at the axon branchpoint, where it colocalizes with synaptic vesicle proteins, such as synaptobrevin-1 and Rab-3. We used a GFP degradation system to deplete KNL-1 specifically in the touch receptor neurons during development. This assay led to axonal misguidance and defects in the formation of the axon branch. A similar post-mitotic function for outer kinetochore proteins has been described in the formation of neuromuscular junction of embryonic motor neurons in Drosophila [1].
Currently we are using biochemical and genetic approaches to understand how KNL-1 affects axon development. Overall, these results have identified a novel role for the kinetochore machinery in axonal branching and synapse formation.

[1] Zhao et al. (2019), *Dev Cell*

321V **Investigating cell type-specific modes of Wnt dispersal *in vivo*** Michelle Favichia[1], Bob Goldstein[2], Ariel M Pani[31]Biology, University of Virginia, [2]University of North Carolina at Chapel Hill, [3]University of Virginia

Wnt proteins are evolutionarily conserved signaling ligands with critical roles in development, homeostasis, and disease states. Wnts often move between cells to signal at a range of distances and have been shown to utilize several mechanisms for cell-bound or free dispersal in different contexts. However, the cell biological processes that mediate Wnt movement between cells *in vivo* are unknown in many cases, and the extent to which differences in Wnt dispersal mechanisms vary by cell type, ligand, and/or organism remain uncertain. We are using *C. elegans* as a tractable system to investigate Wnt dispersal mechanisms in living animals using live imaging of endogenously tagged proteins. Live imaging of two Wnt homologs, CWN-1 and EGL-20 revealed two instances where we hypothesize that neurons, or neuron-like cells, utilize different transport processes to deliver Wnts over long distances. We observed that CWN-1 is expressed in the Canal Associated Neuron (CAN) cells during larval development, and endogenously tagged CWN-1::mNG is transported in mobile punctae that are trafficked along bipolar, axon-like extensions that extend the length of the body. To test the extent to which Wnt transport in neurons may generally resemble CWN-1 transport in CAN cells, we examined EGL-20::mNG transport in *egl-20* expressing neurons with axons that project along the ventral nerve cord. Intriguingly, we did not observe EGL-20::mNG punctae in axons, which suggests the mode of transport in these cells differs from CWN-1 transport in CAN. To explore whether transport mode depends on cell type vs. ligand, we then expressed EGL-20::mNG in CAN cells. Transgenically expressed EGL-20 localized to mobile punctae in CAN cell extensions similar to endogenous CWN-1, which suggests that transport modes for these proteins depends on cell type rather than intrinsic properties of different Wnt ligands.

322V **Anillin and the microtubule bundler PRC1 maintain myosin in the contractile ring to ensure completion of cytokinesis** Inês C Santos[1,2], Ana M Silva[1,2], Reto Gassmann[1,2], Ana X Carvalho[1,21]i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal, [2]IBMC—Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal

Cytokinesis is the last step of cell division, when one cell physically divides into two cells. In animal cells, cytokinesis involves the assembly and constriction of a cortical actomyosin contractile ring that forms during anaphase at the equator of the cell. Contractile ring constriction depends on the generation of force by myosin sliding on the actin filaments. Contractile ring assembly requires signals from an array of antiparallel microtubule bundles (the central spindle) that forms between the two masses of segregating chromosomes. Bundling of central spindle microtubules is essential for cytokinesis in cultured cells. Using a temperature-sensitive mutant of SPD-1, the homolog of the central spindle microtubule bundler PRC1, we demonstrate that SPD-1 is required for robust cytokinesis in the C. elegans early embryo. SPD-1 inhibition results in broadening of the contractile ring, which creates an elongated intercellular bridge between the two sister cells at the last stages of contractile ring constriction that fails to seal. Moreover, depleting the contractile ring component anillin ANI-1 in SPD-1-inhibited cells results in progressive myosin loss from the contractile ring during the second half of furrow ingression, which in turn results in furrow regression and cytokinesis failure. Our results thus reveal a novel mechanism involving the joint action of anillin and PRC1, which operates during the later stages of furrow ingression to re-enforce myosin localization in the contractile ring, ensuring its continued functioning until cytokinesis is complete.

323V **Intermediate Filaments Associate with Aggresome-like Structures in Proteostressed *C. elegans* Neurons and Influence the Rate of Large Vesicle Extrusions as Exophers** Meghan Lee Arnold[1,1], Jason Cooper[1], Rebecca Androwski[1], Sohil Ardeshna[1], Ilija Melentijevic[1], Joelle Smart[1], Ryan Guasp[1], Ken C.Q. Nguyen[2], Ge Bai[1], David H Hall[2], Barth D Grant[1], Monica Driscoll[11]Rutgers University, [2]Department of Neuroscience, Albert Einstein College of Medicine

In human neurodegenerative diseases, toxic protein aggregates can spread between neurons to promote pathology. In the transparent animal model C. elegans, stressed neurons can concentrate fluorescently tagged protein aggregates and organelles and extrude them in large membrane-bound vesicles called exophers, which enter neighboring cells. C. elegans exophergenesis may occur by mechanisms analogous to those that enable aggregate spreading in the human brain in neurodegenerative disease. Here we report on aggresome-like biology in stressed C. elegans neurons that influences exophergenesis. We show that C. elegans intermediate filament proteins IFD-1 and IFD-2 can assemble into juxtanuclear structures with molecular and cellular characteristics similar to mammalian aggresomes and document that these intermediate filaments are required cell autonomously for efficient exopher production. IFD-concentrating structures expand with age or neuronal stress level, can associate with neurotoxic polyglutamine expansion protein HttQ74, and depend upon orthologs of mammalian adapter proteins, dynein motors, and microtubule integrity for aggregate collection into juxtanuclear compartments. IFD homolog human neurofilament light chain hNFL can partially substitute for C. elegans IFD-2 proteins in promoting exopher production, indicating conservation of the capacity of intermediate filaments to influence large neuronal extrusions. In sum, we identify an unexpected requirement for specific intermediate filaments (counterparts of human biomarkers of neuronal injury and disease, and major components of Parkinson's disease Lewy bodies) in large vesicle extrusion from stressed neurons.

324V **Investigating the Regulation of Separase during Cell Division** Christopher Turpin-Sorensen[1], Joshua Bembenek[21]Wayne State University, [2]Mott Center for Human Growth and Development, Wayne State University

Separase is a critical protease that cleaves cohesin to allow chromosome segregation at the metaphase to anaphase transition. Separase also promotes exocytosis during anaphase to coordinate these disparate cellular processes. Separase protease activity is activated at anaphase onset when its inhibitory chaperone, securin, is degraded after ubiquitination by the Anaphase Promoting Complex/Cyclosome (APC/C). We investigated how this canonical pathway regulates separase function in exocytosis. We first evaluated the localization of separase and securin during oocyte meiosis I. In prometaphase I, separase and securin colocalize to chromosome, spindle, and cytoplasmic structures containing kinetochore proteins. In anaphase I, securin is largely degraded by the time that separase moves to sites of action on chromosomes and vesicles. Interestingly, separase does not colocalize with cohesin at the midbivalent region of chromosomes until seconds before cohesin loss and poleward movement of chromosomes. When APC/C is inactivated, separase remains localized to kinetochore-based structures and does not localize to the midbivalent or vesicles. In contrast, partial securin depletion by RNAi causes precocious SEP-1 vesicle localization. Depletion of APC/C and securin is known to have pleiotropic effects besides just affecting separase. Therefore, we overexpressed degron-mutant Securin fused to GFP (Securin^{DM}) in oocytes to determine how it affects separase activity and localization in the presence of normal APC/C activity. Securin^{DM} is stably expressed during anaphase I and causes severe embryonic lethality, chromosome segregation defects, polar body extrusion failures and inhibition of cortical granule exocytosis. Interestingly, expression of Securin^{DM} interferes with separase localization to vesicles but not to the midbivalent and spindle during anaphase I. We hypothesize that Securin^{DM} blocks separase binding to substrates (cohesin and vesicle substrates) but does not block separase interactions with kinetochore proteins known to localize to the spindle. By spatially restricting separase from substrates, the local concentration of this enzyme would be dramatically lower in addition to securin regulation of its activity. Our findings indicate that the canonical cell cycle regulatory pathway controlling separase protease activation also regulates the dynamic subcellular localization of separase to precisely control its activity.

325V **Automated Microscopy Image and Video Analysis Tool for Biological Applications.** Manos ChaniotakisBioMarkerImaging

The analysis of microscopy images and videos is a challenging and time-consuming task. It often requires specialized technical expertise, expensive software, or equipment. Here we present a very efficient open-source tool called Celer Sight^{AI} which allows researchers to perform automated analyses on low-cost hardware without the need for specialized personnel. By selecting the organism and analysis type, Celer Sight^{AI} performs segmentation of all images and generates the appropriate data plots automatically. The Celer Sight^{AI} prototype can generate region-of-interest (ROI) for various *C.elegans* parts of the body, including head, tail and intestine. The supported analysis workflows include fluorescent pixel intensity, particle and colocalization analysis. We also offer a suite of smart interactive tools that allow researchers to label other organisms and regions, accelerating ROI generation with speeds up to ten-fold compared to a traditional polygon selection tool. Celer Sight^{AI} also provides deep learning-based image denoising and super resolution tools to enhance the signal-to-noise ratio (SNR). Finally, Celer Sight^{AI} deep learning model training and deployment pipeline allows for automatic model re-training on a regular basis.

326V **Establishment of *Caenorhabditis elegans* as a Model System in Anthelmintic Drug Discovery from Natural Sources for the First Time in Bangladesh** Nurnabi AHMED[1], Md. ZIM[1], Babul Chandra ROY[2], Muntasir KAMAL[3], Md. Hasanuzzaman TALUKDER[41]Bangladesh Agricultural University, [2]Parasitology, Bangladesh Agricultural University, [3]Department of Molecular Genetics, University of Toronto, [4]Department of Parasitology, Bangladesh Agricultural University

Nematode infections impact human and livestock health mostly in developing nations. Anthelmintic resistance in nematode has become a global issue. *Caenorhabditis elegans* is emerging as a useful model system to uncover anthelmintic potential of novel compounds and offers high throughput screening (HTS) that is not possible with parasitic nematodes. Currently, India is the only country in South Asia that has *C. elegans* labs. In spite of having huge natural reserves of medicinal plants and natural products (NP), this region is far behind in drug discovery research using NP-based HTS platforms compared to other regions of the globe. As a part of an approach to establish *C. elegans* culture in our lab facilities, *C. elegans* wildtype N2 strain was generously received as a gift from the University of Toronto, Canada. Following standard protocols, all the animals were cultured in NGM agar plates. During the culture, the physiology, reproductive biology, feeding behavior and growth rate of *C. elegans* were observed for subsequent maintenance and storage of the worms in -80°C and liquid nitrogen in the lab. In addition to the new experience, abnormalities in growth rate, adaptation to habitat, temperature, feeding habit, effect of bacterial and fungal contaminations and survivability in lab conditions and freezing protocol of the worms were also noted. Preliminary survivability tests were conducted in which the motility of different stages (L1 to adult) of *C. elegans* at 22°C temperature was observed and quantified using commonly-used nematocidal drugs (albendazole, levamisole and ivermectin) at different concentrations (0.1, 0.01, 0.001, 0.0001, and 0.00001 µg/ml) and plant extracts from Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) leaves at different concentrations (1%, 2.5% and 5% w/v) in 96 well plates in controlled environment. The survivability test revealed that ivermectin and levamisole killed all the worms within 10-15 minutes at the higher concentrations whereas more than 30% worms survived in albendazole treated group at the same concentrations. Importantly, no worms survived in both the plant extract treated groups. Bangladesh has plentiful medicinal plants having anthelmintic potency. Therefore, active plant biomolecules from these plants need to be extracted to screen their potentiality in killing nematodes. Therefore, establishment of *C. elegans* will be useful for the discovery of new anthelmintic in this region. We are currently seeking collaborations for isolation and characterization of active plant biomolecules to be tested on this model organism to foster drug discovery.

327V **Determining endosomal pathway specificity for the TBC-2 family of Rab GAPs** ANANYA JANA, Christian Rocheleau
Department of Medicine, The Research Institute of the McGill University Health Centre

The primary function of endosomal network is to receive and sort cargo molecules to their correct destinations. Effector proteins confer specificity to the cargo sorting and transport between diverse endomembrane compartments. The TBC-2 (Tre-2/Bub2/Cdc16) family of Rab GTPase Activating Proteins (Rab GAPs) were identified as an important regulator of endosomal Rab GTPases in *C. elegans*. *In vitro*, the Pleckstrin homology (PH) domain of TBC-2 binds to phosphatidylinositol-4-phosphate (PI4P), but the biological relevance remains elusive. Studies have indicated that PI4P regulates the recycling tubule formation and TBC-2 also acts as a crucial regulator of endocytic recycling. I hypothesize that PI4P recruits TBC-2 to recycling endosomes. PI4 kinases (PI4Ks) maintain the cellular pool of PI4P in endosomal compartments, but a role for a PI4K in *C. elegans* endosome recycling is still unknown. I tested the role of the *C. elegans* Class II (ZC8.6 and C56A3.8) and Class III (Y75B8A.24 and PIFK-1) PI4Ks in endosome recycling and identified ZC8.6 as being required for endosome recycling, making it a candidate regulator of TBC-2. Existing reports indicate that RAB-10, another recycling regulator, controls ARF-6-mediated endosomal $PI(4,5)P_2$ levels during basolateral recycling in *C. elegans*. The distribution of RAB-10 positive puncta towards the apical surface in *zc8.6* mutants signifies how such interaction affects endosome function at the level of lipid phosphorylation. I am generating a PI4P sensor to determine if ZC8.6 regulates PI4P levels on recycling endosomes, if TBC-2 and RAB-10 colocalize with PI4P, if ZC8.6 is required for RAB-10 and TBC-2 recruitment to recycling endosomes. I will use CRISPR to generate mutants for ZC8.6 and other PI4Ks and will investigate the role of PI4Ks in recruiting TBC-2 and RAB-10 to recycling endosomes. The recent identification of mutations in human TBC-2 homolog, TBC1D2B in a rare neurodevelopmental disorder point out to the physiological significance of the TBC1D2B-associated pathways. TBC1D2-regulated pathways are important in E-cadherin degradation to facilitate metastasis during tumor progression. Thus, the significant implications of both TBC1D2 and TBC1D2B in tumorigenesis and neurodegenerative diseases highlight the importance of understanding the mechanics of TBC-2 function and regulation. Thus, my studies might provide insights into how TBC1D2 and TBC1D2B contribute to disease and potential therapies.

328V **KLP-7/Kinesin-13 dependent microtubule organization and dendritic identity in PVD neurons** Swagata Dey[1], Jessica Feldman[2], Anindya Ghosh-Roy[11]Cellular and Molecular Neuroscience, National Brain Research Centre, [2]Stanford University

Polarized structure and composition of the neurons ensures a unidirectional information flow in the nervous system. During polarization, the neurons establish the axonal and dendritic identities often based on their underlying microtubule organization. Molecular mechanisms of axon differentiation have elucidated critical roles of microtubule-stabilizing and bundling molecules. As microtubules undergo phases of growth and catastrophe, it is unclear how microtubule catastrophe factors participate in the neuronal polarization process.

We are investigating the role of microtubule depolymerizing factors in the establishment of axon-dendrite compartmentalization using PVD neurons. These neurons have an elaborate dendritic arbor, a well-defined axon, and a distinct cytoskeletal constitution in their orthogonal branches. KLP-7 is a microtubule depolymerizing motor from the Kinesin-13 family. In a deletion mutant

of *klp-7*, we observed a mislocalization of the axonal cargo, RAB-3 into the primary and higher order dendrites of the PVD neurons, occupying 11% of its branches, unlike 2% in the wildtype. Unlike *klp-7(0)*, the loss of another microtubule depolymerizing factor, *efa-6* did not affect the localization of RAB-3 indicating a specific role of *klp-7* in the axon-dendrite compartmentalization.

Live imaging of EBP-2::GFP in the *klp-7(0)* mutant revealed an increase in the plus-end-out microtubules and a concomitant decrease in the minus-end-out microtubules. Furthermore, the loss of *klp-7* also decreased microtubule dynamics in dendrites. FRAP measurement of GFP::KLP-7 revealed that KLP-7 is more dynamic in the dendrites. Furthermore, we noticed an ectopic accumulation of UNC-44/Ankyrin, an axon initial segment protein in the ectopic neurites originating from the cell body in *klp-7(0)*. These observations indicated that KLP-7-mediated microtubule depolymerization may play an active role in establishing or maintaining dendritic identity.

Overall, this study elucidates the role of microtubule depolymerizing motor KLP-7/Kinesin-13 in establishing checkpoints at the axon-dendrite boundary and dendritic branches to direct the polarized trafficking.

329V **Exploring the roles of patched, dispatched, and hedgehog related genes in the development of unicellular tubes** Nicholas Serra, Meera V Sundaram Genetics, University of Pennsylvania Perelman School of Medicine

Patched and dispatched proteins are famous receptors and transporters for the hedgehog (Hh) family of proteins. Although *C. elegans* possesses an expansive family of patched and hedgehog like proteins, they are not thought to participate in canonical Hh signaling. Instead, there is growing evidence that these proteins affect the organization of the apical extracellular matrix (aECM), a protective layer that coats the exposed surfaces of epithelia. We found that the dispatched and patched related genes *che-14* and *daf-6* are necessary for the growth of the excretory duct tube intracellular lumen and strict localization of the necessary apical protein RDY-2. We hypothesized that *daf-6* and *che-14* might transport Hh like proteins to the growing duct lumen for a non-signaling, structural role. To explore this, we designed fluorescent tagged versions of *wrt-10* and *grl-2*, two Hh like proteins highly transcribed in the embryonic duct cell. We found that *daf-6* and *che-14* individually are not necessary for the localization of WRT-10 and GRL-2. However, we show for the first time that WRT-10 and GRL-2 localize to the aECM, including within the excretory duct and pore lumens. The WRT-10 expression and localization pattern strongly suggests it is a component of the transient pre-cuticle matrix that precedes each cuticle. GRL-2 is a constitutive component of luminal matrix in the excretory duct and pore, as well as the amphid and phasmid socket cells, therefore it may be a tube-specific cuticle component. These data add to growing evidence that the expanded Hh like protein families in *C. elegans* serve diverse structural roles in the apical matrices that line epithelial tissues.

330V **Branched-chain actin dynamics specify apicobasal polarity in the *C. elegans* intestine.** gholamali jafari[1], Liakot Khan[1], Edward membreno[1], Verena Gobel[21]Harvard University, [2]Massachusetts Gen Hosp

Actin is indispensable for many cellular processes, making the analysis of its specific cellular functions difficult. Although a key polarity cue in single-cell yeast, the role of actin in epithelial polarity is largely restricted to ancillary roles in polarized trafficking or cortex modeling, but actin is not thought to specify the positions of polarized membrane domains.

In unbiased *C. elegans* screens, we independently identified the three components of the conserved branched-chain actin machinery as intestinal polarity cues: UNC-60/cofilin, ARX-2/ARP-2/3, and CAP-1/F-actin capping. Depleting each of these components reverses apicobasal polarity on already polarized but still expanding membranes and prevents the establishment of apicobasal polarity in not-yet polarized intestinal cells. Mild depletion of actin, previously considered dispensable for intestinal polarity, copies this polarity phenotype. Branched-chain actin dynamics is furthermore required to specify the polarized distribution of PAR-3, PAR-6 and PKC-3 at the expanding membrane.

We previously found that disrupting intracellular vesicular trafficking similarly interferes with intestinal polarity and results in the same apicobasal polarity conversion phenotype. We hypothesized that actin might affect polarity by providing the cytoskeletal structure that directs intracellular vesicular trafficking during membrane polarization. Indeed, we found that interference with branched-chain actin dynamics affects multiple vesicle populations, including secretory vesicles, that sink to basal cell poles and fail to reach the future apical domain. Furthermore a cytoplasmic F-actin network, as well as the three actin modulators, shift their location from an initial cytoplasmic and pan-membranous to an apical localization during intestinal polarization.

To identify and track the dynamics of a cytoplasmic F-actin network during polarity establishment, we used positional recording and time-lapse imaging of LifeACT::Dendra2 and LifeACT-PA. We found that during polarized membrane biogenesis in the pre-and post-intercalation embryonic intestine, photoconverted LifeACT translocates from the basolateral to the nascent apical domain (lumen) during this domain's coincident biogenesis and positioning.

Our findings support a mode of membrane polarization where actin-directed trafficking acts as an early intracellular polarity cue

that asymmetrically inserts the nascent apical domain into the growing epithelial membrane to partition apicobasal membrane domains and specify membrane polarity.

331V **Visualization of the Biphasic Calcium Wave during Fertilization in *C. elegans* using a Genetically Encoded Calcium Indicator** Katie Toperzer[1], Savannah Brennan[1], David Carroll[2], Eric Guisbert[1], Karen Kim Guisbert[11]Biomedical Engineering and Sciences, Florida Institute of Technology, [2]Biochemistry and Molecular Genetics, Midwestern University

Fertilization is a critical step in development, yet internal fertilization events are notoriously difficult to visualize. Previously, the wave of calcium signaling that accompanies internal fertilization has been visualized using injection of calcium sensitive dyes. However, the technical difficulty and low throughput of this approach has limited its utility.

Here, we have adapted the genetically encoded calcium indicator jGCaMP7s to visualize the moment of fertilization in *C. elegans* using fluorescence. We term this new tool the CaFE reporter for calcium signaling during fertilization in *C. elegans*. The CaFE reporter captures the previously described properties of the calcium signaling wave without significantly impacting physiology or fecundity. Furthermore, no signal is observed in a strain background with a temperature-sensitive mutation in *spe-9* that enables meiotic maturation and ovulation but blocks fertilization. Therefore, the fluorescent signal observed during ovulation by the CaFE reporter is dependent upon sperm fusion during fertilization. This reporter, in the genetically tractable and optically transparent worm, provides a powerful tool to dissect the oocyte-to-embryo transition inside a living animal.

Demonstrating the utility of the CaFE reporter, we examined the timing of the calcium wave relative to ovulation when the oocyte-to-embryo transition (OET) has been perturbed. Interestingly, we found that a prematurely ovulated oocyte displays a delayed calcium wave upon entry into the spermatheca, in stark contrast to normal ovulation events where the oocyte becomes immediately fertilized. Furthermore, we observed calcium waves in oocytes lacking chromosomes. Therefore, the ability to visualize the calcium wave during fertilization has uncovered unexpected new features of meiotic maturation and fertilization competence.

332V **Genetic analysis of the RNAi defective mutants for zinc-binding molecules** Katsufumi Dejima, Shohei MitaniDepartment of Physiology, Tokyo Women›s Medical University School of Medicine

C. elegans employs an exogenous RNAi system that functions systemically, a phenomenon known as systemic RNAi. In this process, RNA silencing signals are taken up by the cell via clathrin-dependent endocytosis, though the molecular mechanisms remain largely unknown. While RSD-3 (a homolog of epsinR: epsin-related protein) plays a role in systemic RNAi and has motifs for binding of phosphoinositides, clathrin, and clathrin adaptor proteins, studies thus far have not revealed any obvious abnormalities in membrane trafficking associated with *rsd-3* mutants. Through a screen for suppressors of the RNAi defective (Rde) phenotype in *rsd-3* mutants, we identified a zinc transporter *zipt-9* as a suppressor gene. Candidates-based screening of deletion mutants for zinc-binding molecules revealed the involvement of rabonosyn-5 (RABS-5), which contains zinc-binding FYVE and zinc finger (ZnF) domains and is thought to act in a clathrin-mediated endocytic events as a RAB-5 effector, in systemic RNAi. Double mutant analyses suggested that RABS-5 and RSD-3 function genetically independent pathways or processes. The rescue experiments using truncated forms of RABS-5 demonstrated that FYVE is required for the RABS-5 function in systemic RNAi, while ZnF is not. The Rde phenotype of rabs-5 mutants is suppressed by zipt-9 mutants, suggesting that the zinc transported through zipt-9 does not exclusively enhance RABS-5 function or that RABS-5 independently functions with ZIPT-9. Our finding that zinc potentially affects exogenous RNAi when either the RSD-3-dependent or RABS-5-dependent pathway is blocked, while RSD-3 and RABS-5 function differently, highlights that the regulation of systemic RNAi by zinc is a multifaceted process.

333V **The role of nucleosome remodeler LET-418 in germline DSB repair** Deepshikha Ananthaswamy[1], Sereen El Jamal[2], Paula M Checchi[3], Teresa W Lee[11]Biological Sciences, University of Massachusetts Lowell, [2]Marist College, [3]Sanford Burnham Prebys Medical Discovery Institute

All cells encounter stress that cause toxic DNA damage like double strand breaks (DSBs), so organisms have evolved multiple overlapping DSB repair pathways to maintain genome integrity. Full DSB repair in germ cells is particularly important, because any remaining genomic damage will cause deleterious mutations for the next generation. DSB repair must function within the context of local chromatin landscapes, including nucleosome positioning. However, the role nucleosome remodeling during meiotic DSB repair remains poorly understood. Mutations in *C. elegans* LET-418/CHD4, the catalytic subunit of the nucleosome remodeling and deacetylase complex (NuRD), cause defects in DSB repair, affecting embryonic survival and overall fertility. These defects worsen when *let-418* mutants are challenged with exogenous DSBs from cisplatin or hydroxyurea. The excess number of mitotic DSBs and persistent recombination intermediates observed in *let-418* mutants phenocopies what occurs in Fanconi anemia (FA) pathway mutants. We are currently exploring the genetic relationship between NuRD components and the FA pathway. These data will define a role for NuRD during germline DSB repair and determine whether nucleosome remodeling is required to generate an accessible chromatin environment for proper DSB resolution.

334V **The AP-1 clathrin adaptor complex differentially regulates LIN-12/Notch signaling in somatic gonad and the vulval precursor cells** Tatsuya Kato[1,2], Olga Skorobogata[1,2], Haojun Zhu[1,2], Edouarda Taguedong[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 highly dependent on the cellular context, thus it is important to further understand its regulation in different tissues and shed light on their pathologies. *C. elegans* vulval development is a well characterized system to study tissue-specific Notch signaling regulation since it is partly controlled by LIN-12/Notch-dependent cell fate specifications in the gonad and epidermis. A somatic gonad precursor cell with low LIN-12/Notch activity is specified to become the anchor cell (AC). AC function is critical for subsequent induction of vulval precursor cells (VPCs), two of which are specified to the secondary fate through high LIN-12/Notch activity. Modulations of LIN-12/Notch signaling cause observable developmental defects such as ectopic secondary fate inductions or lack of VPC inductions called the vulvaless phenotype, enabling genetic analysis of LIN-12/Notch regulators. UNC-101/AP1M1 is a subunit of the conserved clathrin adaptor complex AP-1 which mediates trafficking from the trans-Golgi network to the plasma membrane. We previously reported that double mutants of *unc-101* and the Rab GTPase *rab-7* exhibited ectopic VPC inductions with secondary fate morphologies, suggesting a role of UNC-101 in Notch signaling during vulval development.

To investigate, I analyzed effects of the *unc-101(sy108)* mutant in a *lin-12(n302)* mutant background. *lin-12(n302)* is a weak gain-of-function allele which inhibits AC specification but is insufficient for ectopic secondary fate induction, resulting in a vulvaless phenotype. I observed that *unc-101(sy108)* suppressed the *lin-12(n302)* vulvaless phenotype through restoration of the AC fate, indicating that UNC-101 activity promotes LIN-12/Notch signaling in the AC. Interestingly, *unc-101(sy108); lin-12(n302)* animals also exhibited ectopic secondary fate inductions in the VPCs, suggesting that UNC-101 inhibits LIN-12/Notch in the VPCs. These results point to differential LIN-12/Notch signaling regulation by AP-1 in the somatic gonad and vulva, possibly through tissue-specific sorting of LIN-12/Notch or its regulators. To explore this possibility, I will test if AP-1 regulates LIN-12/Notch localization differently in somatic gonad precursor cells and the VPCs.

335V **Nascent protein synthesis is required for spindle pole re-establishment during MI-MII transition during spermatocyte divisions in *C. elegans*** Szu-Yu Chen[1], Shang Yang Chen[1], Yu-Hao Chen[1], Jui-ching Wu[1,21]Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, [2]Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

Spermatocytes divide with a unique process during which two successive chromosome segregation events take place without apparent pausing. Our laboratory focuses on investigating how the cell cycle programs regulate the repetition of division during spermatogenesis. We previously found that inhibition of proteasome activities prohibited chromosome segregation in MI but not MII spermatocytes, indicating the two division events are regulated through distinct mechanisms. In this study, we aimed to investigate how nascent protein synthesis affects the progression of the male meiotic divisions. We found that primary spermatocytes treated with translation inhibitors could finish the MI division successfully. Nonetheless, the resulting secondary spermatocytes failed to initiate the second division. These results support the hypothesis that primary spermatocytes are loaded with essential factors for the MI division, during which the factors for MII division are generated through nascent protein synthesis. Our results showed that inhibition of translation did not affect the overall kinetochore structure during the MI-MII transition. Nonetheless, the spindle poles failed to re-establish after the completion of the MI division. In primary spermatocytes treated with translation inhibitors, although the centrioles duplicate normally, the centrosomes failed to mature. We found that polo-like kinase PLK-1 failed to localize to the spindle poles after the completion of MI division with translation inhibition. Results from metabolic labeling experiments showed that nascently synthesized proteins did not localize to specific division structures. We hypothesize that the signaling cascade needs to be rebuilt during the spermatogenesis MI-MII transition.

336V **The role of TBC-2 in AKT-1 mediated regulation of HLH-30/TFEB** Soumyendu Saha, İçten Meraş, Christian E RocheleauAnatomy and Cell Biology, McGill University

The activity of transcription factors is regulated by signalling pathways through a variety of post transcriptional modifications including phosphorylation or de-phosphorylation of specific residues. Activated receptors involved in signal transduction pathways are endocytosed into early endosomes then to the late endosome and finally fuse with the lysosome to result in signal attenuation. However, the spatial regulation of transcription factors is not well understood. The DAF-16/FOXO transcription factor is negatively regulated by insulin/IGF signaling pathway. Activated DAF-2/Insulin receptor activates AKT kinases, which phosphorylates DAF-16/FOXO. Phosphorylated DAF-16/FOXO, by interacting with 14-3-3 proteins, is sequestered to the cytoplasm away from the nucleus. We previously reported that DAF-16/FOXO can localize to endosomes in the intestinal cells. Loss of TBC-2, a RAB-5 GTPase Activating Protein involved in RAB-5 to RAB-7 transition during endosome maturation, results in increased localization of DAF-16/FOXO to endosomes at the expense of nuclear localization in the intestine of C. el-

egans. This has effects on DAF-16 target gene expression and Insulin/IGF signalling regulation of longevity and lipid storage. HLH-30/TFEB regulates lysosome biogenesis, autophagy and is involved in longevity in C. elegans. HLH-30/TFEB forms a complex with DAF-16/FOXO and plays a combinatorial role with DAF-16 to regulate an overlapping set of genes in response to certain stresses. We show that in tbc-2(tm2214) mutants HLH-30/TFEB and DAF-16/FOXO co-localize on same endosome. As previously reported for DAF-16, we found that daf-2(RNAi) and akt-1(RNAi) suppressed HLH-30/TFEB localization to endosomes in tbc-2(tm2241) mutants suggesting that TBC-2 might antagonize insulin/IGF signaling. TBC-2 was identified in a yeast two-hybrid screen for CNK-1 interactors (Rocheleau and M. Sundaram, unpublished) and mammalian CNK1 is an Akt scaffold, suggesting that CNK-1 could function with TBC-2 to regulate DAF-16 and HLH-30. We found that cnk-1(RNAi) suppressed the localization of HLH-30 to endosomes in a tbc-2(tm2241) mutant. We hypothesize that CNK-1 promotes insulin/IGF signaling, and that CNK-1 could be negatively regulated by TBC-2.

337V **Role of C9orf72/SMCR-8 protein complex in regulating LET-23 EGFR signaling via the ARF6 GTPase** Ahmed Sabbir, Aida Sobhani, Stephanie Deng, Kimberley Gauthier, Christian RocheleauAnatomy and Cell Biology, McGill University

An expansion of hexanucleotide repeats in the first intron of C9orf72 is the most prevalent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). RNA and protein aggregates resulting from the hexanucleotide repeats and C9orf72 haplo-insufficiency may cause disease. Thus, knowing the normal biological functions of the C9orf72 protein is important to understand how loss of C9orf72 might contribute to disease progression. C9orf72 forms an obligate complex with SMCR8 and they have been implicated in several aspects of endolysosomal and autolysosomal function. Recent structural and biochemical data indicate thatC9orf72 and SMCR8 function as a Rab and Arf GTPase Activating Protein (GAP) *in vitro*. GTPases switch between being in an “active” GTP-bound state and an “inactive” GDP-bound state. GAPs facilitate GTP hydrolysis by GTPases and thus function to switch GTPases into an inactive state. In *C. elegans*, ALFA-1(C9orf72) and SMCR-8 protein complex has been proposed to affect the lysosomal homeostasis. However, the *in vivo* targets of ALFA-1/SMCR-8 have not been identified. We previously demonstrated in *C. elegans* that the ARF-1 and RAB-7 GTPases regulate LET-23 Epidermal Growth Factor Receptor (EGFR) membrane trafficking and are strong negative regulators of LET-23 EGFR-mediated vulva cell fate specification. We tested if ALFA-1 and SMCR-8 might regulate LET-23 EGFR signaling and found that they are also strong negative regulators of vulva induction. ALFA-1 and SMCR-8 are unlikely function with either RAB-7 or ARF-1 as loss of ALFA-1 does not result in LET-23 EGFR trafficking defects. We found that an activating mutation in the ARF-6 GTPase antagonizes LET-23 EGFR signaling and that ARF-6 is required for loss of ALFA-1 and SMCR-8 to increase EGFR signaling. Thus, ALFA-1/SMCR-8 could function as an ARF-6 GAP to regulate LET-23 EGFR signaling. Since ALFA-1 and ARF-6 do not affect LET-23 EGFR localization, we hypothesize that they regulate a transmembrane regulator of LET-23 EGFR signaling such as the DEP-1 receptor protein tyrosine phosphatase.

338V **Identification of neuronal microtubule regulators using forward genetic screening** Sunanda Sharma[1], Dharmendra Puri[2], Keerthana Ponniah[2], Pankajam Thyagarajan[2], Anindya Ghosh Roy[21]Cellular and Molecular Neuroscience, National Brain Research Centre, [2]National Brain Research Centre

Microtubules play an important role in the development and maintenance of the nervous system. It is not clear how microtubules are differentially organized in the dendritic and the axonal compartments of the neuron. We are using *C. elegans* mechanosensory neurons to address these questions. We previously showed that loss of KLP-7, a kinesin-13 family microtubule depolymerizing protein, leads to ectopic neurite extensions in touch neurons, which can be suppressed by the pharmacological destabilization of microtubules (Puri et. al., 2021). We hypothesized that a forward suppressor genetic screen in *klp-7* mutant might help identify some novel regulators of the neuronal microtubule cytoskeleton. We isolated 26 suppressors from 12,422 F1s in a clonal EMS mutagenesis screening in *klp-7(0)* background. We mapped these suppressors by combining whole genome sequencing and EMS density mapping using MimodD tools on the Galaxy user interface.

About nine suppressors mapped either into *mec-7 (b-tubulin)* or *mec-12 (a-tubulin)* gene. Interestingly one of the suppressors was mapped to *muscleblind-1/mbl-1* gene. MBL-1 is a muscle-blind family protein that regulates alternative splicing, RNA stability and RNA localization. Mutations in *mbl-1* have been linked to neuromuscular disorder myotonic dystrophy. We found that loss of *mbl-1* affects microtubule dynamics and axonal transport in touch neurons. Moreover, we found that MBL-1 regulates the stability of *mec-7* and *mec-12* mRNA transcripts in these neurons.

Another suppressor was mapped to *W02B8.2/citk-1,* an ortholog of mammalian citron kinase gene. Mutations in citron kinase have been associated with microcephaly in humans. Worms have another paralog of *citk-1,* which is *F59A6.5/citk-2*. We observed that loss of either of these genes could independently suppress *klp-7(0)* phenotype. We also observed that loss of either of citron kinases leads to morphological defects in the PLM mechanosensory neurons of *C. elegans*. The anterior process of PLM neuron is wavy in mutant with occasionally also forming an ectopic branch. Using the plus-end binding EBP-2::GFP reporter, we found that the microtubule dynamics is upregulated in the *citk-1* mutant.

We will summarize our findings on *mbl-1* and citron kinase mutations and present a comprehensive view of the screen.

Reference:

Puri, D., Ponniah, K., Biswas, K., Basu, A., Dey, S., Lundquist, E. A., & Ghosh-Roy, A. (2021). Wnt signaling establishes the microtubule polarity in neurons through regulation of kinesin-13. *Journal of Cell Biology*, *220*(9). https://doi.org/10.1083/jcb.202005080

339V **Lysosomal copper transporter in pharyngeal muscle underlies cold tolerance of *C. elegans*** Serina Yamashiro, Satomi Mizuno, Atsushi Kuhara, Akane Ohta, Haruka MotomuraGraduate school of Natural Science, Konan University

We are using the cold tolerance of nematode *C. elegans,* as a model for studying animal temperature acclimatization. To isolate new molecules for cold tolerance, we used EMS mutagenesis and deep sequencing analyses and revealed that the gene responsible for the cold tolerance mutant was *slcr-46.1* that encodes a homolog of a human steroid conjugate and bile acid transporter SLC46A3. Human SLC46A3 localizes in the lysosomal membrane, but it's *in vivo* function is largely unknown. Antibody staining examined that *C. elegans*SLCR-46.1 localized into lysosomes, similar to human SLC46A3. The SLCR-46.1 was expressed in the pharyngeal muscle, intestine, cuticle, and alae. However, *slcr-46.1* cDNA was also expressed in ASG and BAG sensoryneurons, which are cold tolerance-related neurons, suggesting that intron of *slcr-46.1* gene inhibits its neuronal expression. A tissue-specific rescue experiment demonstrated that the cold tolerance abnormality of *slcr-46.1* mutant was caused by the impaired pharyngeal muscle. Besides, rescue lines expressing SLCR-46.1 specifically in the pharyngeal muscle, ASG, and BAG had significantly lower survival rates than rescue lines only expressing SLCR-46.1 in the pharyngeal muscle. This suggests that SLCR-46.1 expressed in neurons suppresses cold tolerance. We found that the number of pharyngeal pumping in the *slcr-46.1* mutant was decreased. We are now attempting to quantify the lysosomal activity in the pharyngeal muscle of *slcr-46.1* mutant using an optical indicator, to analyze the relationship between lysosomal activity and cold tolerance.

340A **A comprehensive high-resolution, high-throughput screening platform reveals cell invasion defects in *C. elegans*** Simon Berger[1,2], Silvan Spiri[1], Evelyn Lattmann[1,3], Mitchell Levesque[3], Andrew deMello[2], Alex Hajnal[11]Department of Molecular Life Science, University Zurich, [2]Institute for Chemical- and Bioengineering, ETH Zurich, [3]Department of Dermatology, University Zurich

Traditionally, immobilization of *C. elegans* during image acquisition is accomplished using agar-pads. However, immobilization in this manner is known to be invasive, resulting in a slowdown or complete arrest of development. Furthermore, preparation and use of agar-pads is time-consuming and tedious, especially during large-scale experimentation. We therefore present a new, simple to use, high-throughput, high-resolution *C. elegans* imaging method using the imaging devices introduced in Spiri *et al.*, along with fully automated high-speed image acquisition and data analysis.

Worm immobilization is achieved in a microfluidic device consisting of a parallel trap channel array, allowing simultaneous imaging of multiple animals in a single field of view, and up to 50 animals in a device unit. Throughput is further increased by housing several units in one device, trapping up to 400 animals simultaneously. Animals are loaded quickly and easily, without need for any specialized equipment. Channel geometry is designed closely to the size and shape of the animals at the desired developmental stage, and immobilization is achieved entirely passively. Animals on-chip are reliably oriented, such that no time is spent locating trapped worms. Currently eight different devices are available (mid-L2 to two-day-old adults). All devices are imaged fully automatically using custom build microscope control software, compatible with high magnification, high numerical aperture objectives (up to 60x), and all common imaging modalities (DIC, epifluorescence, confocal). Combined our fully automated screening platform allows accelerated image acquisition at a pace and resolution not achievable with other system, all while remaining inexpensive and simple to use.

The method was validated through an RNAi screen identifying the role of 193 genes in basement membrane (BM) breaching during anchor cell invasion. Approx. 40'000 animals at the L3 to L4 molt were imaged at 40x magnification and a throughput of 1200 worms/hr. Images were then analyzed using a convolutional neural network, trained to differentiate between normal and inhibited BM breaching. All animals were scored fully automatically, achieving an average scoring accuracy of more than 90%. In total, we identified 92 genes affecting BM breaching, 51 positive controls included in our library as well as 41 genes not previously identified in the context. See accompanying abstract Kao *et al.* for method applications in drug screening.

Spiri *et al.* 2022. Reciprocal EGFR signaling in the Anchor Cell ensures precise inter-organ connection during C. elegans vulval morphogenesis. *Development*. 149, 199900.

341A **Eggshell contributions to late embryogenesis in *C.elegans*** Akiko Hatakeyama, Shuichi OnamiRIKEN Center for Biosystems Dynamics Research

Extracellular matrices covering the apical surface of epithelia (aECMs) protect cells from external environments and shape tissues during late embryogenesis. Different types of aECM surround embryonic cells, and these functions still need to be fully understood. Here, we show that the outermost aECM in *C.elegans* embryos, or the eggshell, contributes to regulating both microstructure formation on the body surface and starvation resistance in newly hatched larvae (L1s). To investigate the functions of the eggshell, we removed the eggshells from eggs and inspected the hatched L1s from various aspects. The eggshell removal caused dumpish body shapes, shorter body length, and defects in microscale lateral ridges, called alae, on the surface coat of the L1s› body. These larvae also decreased resistance to starvation; after six days of starvation, only 24% of the L1s grew into adults when fed instead of about 90% recovery for intact L1s. These results suggest that the eggshell affects developmental processes, including body shaping, microstructure formation in the cuticle, and signaling pathways in stress resistance, indicating more diverse functions of the eggshell than previously thought. To identify when and how the eggshell contributes to developmental processes, we analyzed eggshell effects on events at late embryogenesis. During the stages of body shaping, embryos without eggshells elongated their body normally regarding elongation speed, actin-fiber orientation in epidermal cells, and seam cell dynamics. We are now focusing on the stage of cuticle formation, or the last stage of embryogenesis, and looking at eggshell effects on several processes related to cuticle formation and stress resistance. We speculate that the eggshell controls diverse processes in the last stage of embryogenesis for the proper transition from embryo to larva by regulating gene expression patterns or molecular localization. Since every other animal embryo has some aECMs at interfaces with external environments, this study may contribute to understanding the common functions of aECMs in regulating late embryogenesis.

342A **Unraveling the regulatory network of a complete C. elegans neural lineage** Euclides E Fernandes Povoa, Annabel Ebbing, Marco Betist, Lucia Garcia del Valle, Rik KorswagenHubrecht Institute

Nervous system development comprises the generation, positioning and connection of diverse types of neurons in order to form highly organized neural circuitries. Our current knowledge shows that transcription factors (TFs) govern the molecular mechanisms that underlie different neural development stages such as progenitor cell proliferation, cell migration and lineage commitment. However, comprehensive in vivo characterization of such molecular regulation in a complete neuronal lineage is still scarce. In the nematode C. elegans, the QR neuroblast lineage arises from a stem cell-like progenitor and undergoes a long anteroposterior migratory process to give rise to three final neuronal descendants: two sensory neurons (AQR and AVM) and one interneuron (SDQR). Th entire lineage - from progenitor to final descendants - encompasses a total of nine cells generated by four cell divisions. We are using this single cell model system to deepen our knowledge on how a complete neuronal lineage -including its migration and differentiation into different neuronal subtypes - can be regulated at the transcriptional level. We have combined FACS-based Q neuroblast sorting and RNA-sequencing (CEL-seq) to better understand the temporal transcriptional dynamics occurring in the QR lineage. Based on the data generated, we have identified hundreds of TFs that are expressed in this lineage. We selected a list of candidates that were upregulated in the QR lineage compared to the progenitor cells, and which we are currently characterizing for their roles in neural lineage commitment and progression, neuroblast migration, and neuronal differentiation.

343A **Connecting vulva cell fate specification to apical extracellular matrix organization** Helen F Schmidt, Meera V SundaramGenetics, University of Pennsylania

The interior of biological tubes is shaped and maintained by the apical extracellular matrix (aECM). Proteins found in aECMs include collagens, mucins, and Zona Pellucida domain (ZP) proteins and are often organized into distinct spatial domains. Our lab found that the developing *C. elegans* vulva contains a complex luminal aECM with distinct domains that are associated with specific cell types. The organization of the vulva aECM is not well explained by the transcription pattern of matrix protein genes. Instead, we hypothesize that each cell type expresses different matrix organizing factors that recruit its unique set of aECM proteins.

The vulva is a well-established model for regulatory networks and tubulogenesis with only 22 cells: the EGF-Ras-ERK induced primary (1˚) cells (vulE/F) and the Notch induced secondary (2˚) cells (vulA/B1/B2/C/D). 1˚ cells recruit the ZP domain of LET-653 (LET-653(ZP)) to their surface, while specific 2˚ cells recruit ZP protein NOAH-1 or secreted eLRRon SYM-1. Additional proteins localize to different surfaces depending on the developmental stage or form structures in the center of the vulva lumen, distinct from any cell surface.

We tested whether mutants in transcription factors required for different markers of vulva cell fate affected the localization of fluorescently tagged NOAH-1 and LET-653(ZP). We found that the paired-box transcription factor EGL-38 is required to recruit LET-653(ZP) to the 1˚ cell matrix. In contrast, ETS transcription factor LIN-1 is not required for LET-653(ZP) localization, and the LIM homeobox transcription factor LIN-11 is not required for either protein's localization. Our ongoing work will use single-nucleus RNA-Seq of sorted vulva nuclei to identify candidate matrix organizers with cell type specific expression.

344A **Coordination of pharynx and body growth by an ultra-sensitive coupling via *yap-1*** Klement Stojanovski[1], Ioana Gheo-

rghe[1], Anne Lanjuin[2], William B Mair[2], Benjamin D Towbin[11]University of Bern, [2]Harvard T.H. Chan School of Public Health

The growth rate of cells and organs is subject to environmental and stochastic variation. In principle, such fluctuations could add up to large differences in organ size proportions during development. It is not understood why the size differences between individuals nevertheless remain very small. By live imaging of hundreds of individuals throughout post-embryonic development, we reveal a precise control of size proportions for the pharynx of *C. elegans*. Small pharynxes grew faster than larger ones and caught up in size during development. Moreover, pharynx-to-body size proportions were robust even to strong experimental perturbation in growth rates by tissue-specific inhibition of mTORC1 and insulin signalling. Tissue-specific inhibition of growth triggered a systemic response that coordinated the growth of all body parts. Mathematical modelling showed that this response requires a bi-directional ultra-sensitive coupling between body growth and pharynx size that cannot be accounted for by changes in food intake alone. Indeed, an RNAi screen revealed an involvement of molecular regulation. Knock-down and deletion of the mechano-transducing transcriptional co-activator *yap-1*/YAP perturbed systemic growth coordination, such that tissue-specific inhibition of mTOR resulted in continuous divergence of the pharynx from its appropriate size. We conclude that mechanical signals coordinate the growth of different organs of *C. elegans* with each other to ensure the faithful development of appropriate size proportions.

345A **Two distinct myosins contribute to early vs late stages of spermatheca organogenesis** Fung Yi Chan, Daniel Sampaio Osório, Reto Gassmann, Ana Xavier CarvalhoInstituto de Biologia Molecular e Celular (IBMC), Instituto de Investigação e Inovação em Saúde (I3S)

The *C. elegans* spermatheca is a contractile myoepithelial tubular organ that oocytes have to traverse to be fertilized by sperm and constitutes a powerful model to understand organogenesis. The motor known to drive spermatheca contractility is the non-muscle myosin 1 (NMY-1). In this study we discovered that the other *C. elegans* non-muscle myosin, NMY-2, is also expressed in the spermatheca but only contributes to the early stages of spermatheca development. Throughout development of the organ, NMY-2 levels progressively decrease, while NMY-1 levels progressively increase. This transition is accompanied by a change in actomyosin dynamics from a pulsatile network to stabilized stress-fiber-like actomyosin bundles, characteristic of the adult spermatheca. Selective depletion of NMY-2 demonstrated that NMY-2 ensures the early cell divisions that give rise to the 24 myoepithelial cells that form the spermatheca and contributes to priming bundle formation, but not for bundle stabilization. In contrast, NMY-1 is dispensable for cell divisions but absolutely essential for bundle formation and stabilization. Forced expression of NMY-2 during late spermatheca development using the promoter and 3'UTR of NMY-1 revealed that NMY-2 is unable to form bundles and cannot replace NMY-1 function when NMY-1 is depleted. Interestingly, we found that a chimera of NMY-2 head and NMY-1 tail, expressed under the promoter and 3'UTR of NMY-1, can form bundles and functionally replace NMY-1. We therefore conclude that distinct myosins associate with distinct spermatheca developmental stages, and distinct actin network dynamics. Moreover, our findings reveal the specialization of NMY-1 tail on contractile bundle formation and stabilization.

346A **Validation of single cell transcript numbers with smiFISH** Vincent Portegijs, Alexander Blackwell, Molly Godfrey, Sander Van den HeuvelDevelopmental Biology, Utrecht University

During organismal development, most somatic cells undergo differentiation and contribute to the formation of specialized tissues. The process of differentiation is controlled at many levels, in particular through differential gene expression. The emergence of next-generation sequencing techniques such as single-cell RNA sequencing (scRNA-seq) has allowed detailed characterizations of the gene expression-patterns associated with adopting specialized cell fates. However, these large datasets do not provide absolute quantifications of mRNA copy number; this requires the use of orthogonal techniques. We employed single molecule FISH (smiFISH) to quantify individual mRNA transcripts in cells of the developing mesoblast (M) to verify data obtained from cell-type specific RNA-seq experiments. Our results show that transcripts of the cell cycle regulator *cyd-1* sharply increase as the cell prepares to divide and diminish when these cells exit the cell cycle. This contrasts our RNA-seq data, which suggested that *cyd-1* transcript levels are already elevated in the quiescent M cell. Through further analysis of single mRNA transcripts from a number of control genes, we find a similar trend, where RNAseq analysis of the earliest developmental timepoints suggests an exaggeration of transcript counts compared to smiFISH. We postulate that this can partly be explained by decreased levels of total mRNA in the starved animals, which impacts the subsequent normalization of RNA sequencing datasets. This study shows the value of combining large-scale sequencing efforts with orthogonal approaches, such as smiFISH, to quantify the expression of candidate genes of interest.

347A **Very old doubles with bubbles: *daf-2(e1370)* / peroxisomal beta oxidation double mutants are very long lived and contain extremely large lipid droplets** Andreas H Ludewig, Diana C Fajardo, Yan H Yu, Camila C Pulido, Frank C SchroederSchroeder lab, Boyce Thompson Institute

The Insulin like signaling (ILS) pathway and the peroxisomal beta oxidation (PBO) are highly conserved metabolic gateways that

regulate the uptake of glucose and amino acids and the breakdown of fatty acids in all animals. ILS has been widely associated with lifespan control across species[1]; PBO has not been directly connected with lifespan changes, but our and other laboratories work showed that small molecular products of *C. elegans* PBO can trigger pro-health and anti-ageing effect (ascarosides #2, #3[2], #8, ncas#3).

We found that double mutants of the *daf-2(e1370)* strain that lacks the tyrosine kinase domain of the insulin receptor with strains mutant for individual members of the PBO enzymatic cascade – *daf-22*, *dhs-28* and *maoc-1* - live significantly longer than *daf-2(e1370)* mutants alone. From young adult stage on, these mutants contain unusual, very large droplets; lipid analysis revealed an increased triglyceride content in one of the mutants. A triple mutant of *daf-2; maoc-1*; and the lipid droplet marker *dhs-3*::GFP revealed that these structures are indeed very large lipid droplets. We will present a set of novel compounds - derived from comparative metabolomic analyses - that are significantly up or down in PBO/ILS double mutants.

We found that simultaneous knock down of two of the most essential and conserved genetic pathways does not kill the animals but instead, increased their lifespan to a remarkable extend. PBO/ILS double mutants produce an overflow of functionally uncharacterized compounds, some of which might directly contribute to the unusual long lifespan of these mutants. Integration of genomic, proteomic and metabolomic data acquired from these mutants will facilitate the creation of a *C. elegans* pathway map of age-related small molecules.

1 Kenyon C., Nature. 2010 Mar 25;464(7288):504-12. doi: 10.1038/nature08980.

2 Ludewig A.H. Proc Natl Acad Sci U S A. 2013 Apr 2;110(14):5522-7. doi: 10.1073/pnas.1214467110.

348A **Autonomous and non-autonomous regulation of somatic gonad and germ line development by TORC1** Julia Wittes, Iva GreenwaldBiological Sciences, Columbia University

Development requires timing and coordination among diverse tissues. In *C. elegans*, the heterochronic gene network controls the molting cycle and stage-specific developmental events for most somatic tissues; however, developmental progression of the somatic gonad and germ line is regulated largely independently of the heterochronic gene network (Rougvie & Moss 2013). Within the gonad, there is coupling of somatic gonad and germline progression; interactions between the somatic gonad and germ line are modulated by environmental conditions during continuous development (Schedl & Hubbard 2019), and in states of arrested development, including dauer diapause (Baugh & Hu 2020). How developmental progression of the somatic gonad is coordinated with the nongonadal soma, and if the nongonadal soma provides input directly to the germ line independent of the somatic gonad, are largely unknown.

To start addressing these questions, we are manipulating the activity of TORC1, an established mediator of responses to nutrient levels and growth factors (Blackwell et al. 2019). In favorable conditions, TORC1 functions as a global regulator of developmental progression: AID-TIR1-mediated depletion of the TORC1 component DAF-15/Raptor in L1-L3 leads to developmental arrest after the next molt (Duong et al. 2020). While global depletion of DAF-15 causes arrest at ~L2 (as in Duong et al.), depletion of DAF-15 in the hypodermis causes global arrest at ~L3, with a corresponding arrest of the somatic gonad and germ line. Global depletion of DAF-15 with concomitant restoration of DAF-15 to the somatic gonad does not appear to restore progression to the somatic gonad or germ line, implying that the nongonadal soma normally cues the gonad to progress. However, depletion of DAF-15 specifically from the somatic gonad causes abnormalities in both the somatic gonad and germ line, implying a role for TORC1 in the somatic gonad as well. Depletion of DAF-15 in other nongonadal tissues does not cause global or gonadal developmental arrest.

We are using targeted and unbiased RNAi screens, globally and tissue-specifically, to identify other genes and networks that couple gonadal with non-gonadal development, and somatic gonad with germline development. Our screens will test the role of TORC1 and identify genes that regulate gonadal progression in dauer larvae (cf. Tenen & Greenwald 2019) and during continuous development.

349A **The role of VAB-9 in the regulation of actomyosin and epidermal morphogenesis** Jonathon Heier[1], Jeff Simske[2], Jeff Hardin[11]Department of Integrative Biology, University of Wisconsin-Madison, [2]Simallier Biomedical Enterprises

Epithelial cell-cell junctions play a crucial role in adhesion, cytoskeletal coordination, and morphogenesis in the developing embryo. The major protein complex present at epithelial junctions is the cadherin-catenin complex and its associated proteins. One of these associated proteins is the four-pass transmembrane protein VAB-9, a member of the claudin family and homologous to mammalian TMEM-47. TMEM-47 has been found to be a marker of Ewing family sarcomas and also upregulated in chemotherapy resistant hepatocellular carcinomas. *C. elegans* embryos lacking VAB-9 develop severe body shape defects during elongation and adults exhibit egg-laying, body morphology, and tail defects. Despite its importance, little is known about the function of VAB-9.

We show that endogenously tagged VAB-9 is initially expressed during dorsal intercalation at epithelial junctions and its proper localization is dependent on the cadherin HMR-1. We hypothesize that body shape defects that result in embryos lacking VAB-9 are due to localized failure of elongation. Elongation requires the coordinated contraction of circumferential actomyosin filament bundles in the epidermis to properly complete this process. The contraction of non-muscle myosin in these bundles is activated by the phosphorylation of the myosin regulatory light chain by the kinase LET-502/ROCK, and inhibited by the myosin phosphatase MEL-11. In wild-type embryos, MEL-11 is recruited to epithelial junctions and co-localizes with VAB-9 to coordinate contraction. In VAB-9 null mutant embryos, however, circumferential actin filament bundles become disorganized and a subset are not parallel as they are in wildtype. In addition, in the absence of VAB-9, MEL-11 does not properly localize to cell-cell junctions. These results indicate that VAB-9 is important for both actomyosin organization as well as the regulation of tension through proper MEL-11 localization, thereby promoting elongation during *C. elegans* development.

350A **Studying chromatin regulation at single-cell resolution during *C. elegans* postembryonic development** Alexander R Blackwell[1], Christian Huisman[1], Peter Zeller[2,3,4], Alexander van Oudenaarden[2,3,4], Sander van den Heuvel[11]Utrecht University, [2]Oncode Institute, [3]Hubrecht Institute-KNAW, [4]University Medical Center Utrecht

Despite containing identical genomes, developing cells differentiate into a plethora of diverse cell types. Underlying this diversity is a complex network of gene expression programmes, and transcriptional readouts now form the basis for classifying cell types. However, it remains unclear how the combinatorial activity of transcription factors, chromatin regulators and epigenetic modifications achieve the proper spatiotemporal patterns of gene expression. Moreover, developmental biologists increasingly appreciate the need to investigate gene regulation at the single-cell level because much heterogeneity and complexity is lost when averaging across populations of cells.

Adapting single-cell methods to profile TFs, histone modifications and transcriptomes in *C. elegans* will provide a powerful toolkit for studying the (epi)genetic regulation of development. To this end, we have established three methodologies: i) chromatin immunocleavage with sequencing (ChIC-seq), ii) chromatin endogenous cleavage with sequencing (ChEC-seq), and iii) transcriptome + ChIC (T-ChIC) for profiling both the transcriptome and a histone modification from the same single cell. All three methods target a micrococcal nuclease in order to induce local nuclease activity – either in the vicinity of a histone modification ((T-)ChIC-seq) or adjacent to a DNA binding protein (ChEC-seq) – with the subsequent DNA break ends being incorporated during sequencing library preparation. All three approaches produce reproducible profiles with a high signal:noise ratio.

We combine Cre/Lox lineage tracing with cell isolation and cell sorting procedures in order to isolate postembryonic mesoblast descendants. Following prolonged quiescence, the mesoblast resumes proliferation and produces fourteen body wall muscle cells, two scavenger cells, and two migratory bipotent myoblasts over 24-hours. By profiling multiomic parameters at high temporal resolution across this developmental time-course, we aim to reveal regulatory processes controlling cellular proliferation and differentiation. This work sheds light on how epigenetic modifications contribute to cellular decision making in a living animal.

351A **A high throughput platform for the elucidation of bacterial species and mechanisms that regulate insulin signaling** Kelsie M Nauta[1], Xiao Wang[1], Darrick Gates[1], Jason Cooper[2], Kim Nguyen[1], Daisy Fu[3], Scott Givan[3], Ryan Sheldon[4], Nicholas O Burton[11]Epigenetics, Van Andel Research Institute, [2]Epigenetics, Van Andel Research Institution, [3]Bioinformatics and Biostatistics, Van Andel Research Institute, [4]Mass Spectrometry, Van Andel Research Institute

Animal microbiomes have been proposed to influence nearly all aspects of animal physiology ranging from development to metabolism to behaviour. Despite these findings, many of the mechanisms by which bacteria influence host physiology remain unknown. It has been argued that "in order to realize its potential, the field needs to focus on establishing causation and molecular mechanism, with an emphasis on phenotypes that are large in magnitude, easy to measure, and unambiguously driven by the microbiota" (Fischbach, Cell 2018).To address this, we have developed and validated a high throughput platform to identify the mechanisms that bacteria use to modulate animal insulin signaling using *Caenorhabditis elegans*. Here, we report the use of this platform to identify three bacterial species (*Pseudomonas lurida, Lysinibacillus sp.* BURb01, and *Bacillus sp.* BURb03) capable of restoring normal development to adulthood in insulin signaling mutants (*daf-2, akt-1*) that otherwise arrest as dauers. These effects on animal physiology are especially robust for some species, such as *Bacillus sp.* BURb03, where we found that 100% of *daf-2* mutants feeding on *Bacillus sp.* BURb03 develop to adulthood under conditions where 0% of *daf-2* mutants develop to adulthood when fed *E. coli* OP50.

To identify bacterial genes required to restore animal development, we conducted a Tn5 transposon mutagenesis in one of our isolates, *Pseudomonas lurida*. We found that a Type VI secretion system (T6SS) and a predicted secreted protein are required for *P. lurida's* effect on *C. elegans* dauer development. To our knowledge, these findings are the first to demonstrate the T6SS of a commensal organism may play a role in modulation of host metabolism in any animal. By contrast, *Lysinibacillus* and *Ba-*

cillus species do not have T6SSs. Consistent with this, we have used fractionation-based approaches to determine that *Bacillus sp.* BURb03 likely mediates its effect on *C. elegans* via a secreted small molecule.

These findings are consistent with our hypothesis that diverse bacteria have evolved mechanisms to promote animal insulin signaling and validates our platform as a method for identifying these species. In the long-term, we expect that the identification of these species and mechanisms will lead to new therapeutic strategies to treat insulin related pathologies.

352A **Peroxisome critically controls the developmental fate by relaying the intestinal nutrient signal to the brain** Na Li, Beilei Hua, Huanhu ZHUShanghaiTech University

Peroxisome is an organelle known for synthesizing many metabolites, but its physiological role was largely enigmatic. In a genetic screen, we surprisingly found that the peroxisome function was essential to maintain the L1 diapause that is controlled by the lipid/TORC1-mediated nutrient-sensing pathway we have identified recently. More interestingly, we found prolonged starvation or deficiency of the lipid/TORC1 pathway relocated intestinal peroxisomes to the apical membrane by kinesin/microtubules, which facilitates the secretion of peroxisomal beta-oxidation derived hormone ascarosides. Those ascarosides further function on their receptor DAF-38 at the ciliated sensory neurons and downstream daf-12 pathway to arrest the L1 development. Our finding suggests peroxisome play a pivotal role in nutrient-dependent developmental fate determination.

353A **A sperm–oocyte protein complex as an actin regulator required for completion of female meiosis, egg activation, and the block to polyspermy** Tatsuya Tsukamoto[1], Ji Kent Kwah[2], Naomi Courtemanche[1], Andy Golden[3], David Greenstein[1], Aimee Jarmillo-Lambert[21]Genetics, Cell Biology, and Development, University of Minnesota, [2]Department of Biological Scien, University of Delaware, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

In most animals, fertilization triggers the process of egg activation, which results in the completion of female meiosis and launches the oocyte-to-embryo transition. *C. elegans spe-11* is one of a few paternal-effect lethal genes. We report that SPE-11 forms a protein complex with an oocyte protein, OOPS-1 (Oocyte Partner of SPE-11), that is required for many post-fertilization events. We uncovered *oops-1* as an mRNA target of the oogenic LIN-41 and OMA-1 RNA-binding proteins. Affinity purification of OOPS-1 protein complexes followed by mass spectrometry identified SPE-11. Expression of both proteins in *E. coli* yields a stable complex, which we purified. We thus compared *spe-11* and *oops-1* mutant phenotypes. Prior analysis of *spe-11(hc90* W191stop*)* embryos showed that oocyte meiotic chromosomes segregate at anaphase I and II but fail to form polar bodies (McNally and McNally, 2005). We found that *oops-1* null mutant embryos exhibit a related phenotype—approximately half display meiotic arrest in meiosis I and meiosis II, with the spindle drifting away from the cortex; whereas, the other half display the *spe-11(hc90*) phenotype. Because genetic results suggest that *spe-11(hc90)* may be a hypomorph (L'Hernault et al., 1988), we deleted the entire SPE-11 ORF and found that *spe-11(tn2059)* null mutant embryos phenocopy *oops-1* null mutants. McNally and McNally (2005) noted that *spe-11(hc90)* mutant embryos exhibit similarities to embryos treated with the F-actin inhibitor latrunculin. Thus, we tested whether the OOPS-1–SPE-11 complex modulates actin assembly. Initial experiments show that the complex inhibits formin-mediated actin assembly *in vitro*. Thus, the OOPS-1–SPE-11 complex may function in part by mediating key meiotic roles of F-actin. Previously, Browning and Strome (1996) reported that SPE-11 supports embryonic development when expressed in the maternal germline. We replicated this result using new tools and found that females expressing mScarlet::SPE-11 in the maternal germline produce 96% viable embryos when mated with *spe-11* null mutant males. This finding raises the question of why evolution has separated OOPS-1 and SPE-11 into separate gametes. A potential answer is suggested by the observation that *spe-11* null mutant hermaphrodites expressing mScarlet::SPE-11 in the male and female germline exhibit transgenerational low fertility and slow growth phenotypes, indicating a potential role in paternal epigenetic inheritance.

354A **The cell cycle regulator APC/C^{FZR-1} controls the decision between the SS and DTC cell fate in Caenorhabditis elegans** Adrian Fragoso-Luna[1], David Borrego[2], Jose Perez-Martin[1,31]Instituto de Biologia Funcional y Genomica (CSIC), [2]Instituto de Biomedicina de Valencia (CSIC), [3]Instituto de Biomedicina de Valencia CSIC

Proper coordination between cell division and differentiation is essential during the development of organisms. Many components of the regulatory network involved in the cell cycle control can act directly on differentiation factors. Among these cell-cycle components highlights the E3 ubiquitin ligase APC/C$^{Cdh1/Fzr1}$.

We have explored the role of APC/C^{FZR-1} during *C. elegans* development. We have obtained a complete loss-of-function allele of *fzr-1*. From the analysis of this mutant, we have found that APC/C^{FZR-1} participates in the development of the somatic gonad, an organ that supports germline development. Specifically, APC/C^{FZR-1} is necessary to produce the Distal Tip Cell (DTC), a stem-cell niche that maintains a pool of germ cells and leads the outgrowth of the gonad. The absence of FZR-1 makes cells committed to being DTCs, acquire the SS precursor fate of sister cells.

Moreover, an allele of *fzr-1* constitutively active yields extra DTCs. Both results suggest that APC/C^{FZR-1} is part of a balance during

the fate choice decision ‹DTC-SS› in the somatic gonad. Forced expression of the transcriptional factors involved in determining the DTC fate, LIN-32 and HLH-12 suppressed the requirement of APC/C^{FZR-1} during DTC determination, suggesting the involvement of a transcriptional regulator as a target of APC/C^{FZR-1}. To search for this target, we carried out an RNAi search, looking for putative targets of APC/C^{FZR-1} involved in this developmental decision. We will discuss obtained candidates and their proposed roles in the process.

355A **Post-transcriptional regulation of PDE-2: a key molecule regulating cAMP and cGMP signalling in *C. elegans*** Jagannath Jayaraj, Kamal Kishore, Spatika Jayaram, Kavita Babu, Vandita D BhatCentre for Neuroscience, Indian Institute of Science

Secondary signalling involving cAMP and cGMP are crucial for spatiotemporal regulation of signalling mechanisms that are required for normal development of an organism, memory formation and synaptic plasticity among multiple other functions. Hence, these pathways must be tightly regulated at multiple levels. RNA binding proteins (RBPs) are a class of proteins that post-transcriptionally regulate gene expression by modulating the stability of the mRNA to which they bind. In this work, we explore the RBP-mediated post-transcriptional regulation of PDE-2 (Phosphodiesterase 2), a key molecule in the secondary signalling pathway through cAMP and cGMP. Using yeast-three-hybrid assays and EMSA experiments we show that the RBP protein belonging to the PUF protein family binds directly to the 3' UTR of PDE-2 and that this interaction is required for the egg-laying function of PDE-2. Furthermore, mutating the binding site at the 3' UTR of PDE-2, hence preventing PUF binding to the 3'UTR of PDE-2 mimics multiple defects seen in *pde-2* mutants including the egg-laying defects.

Our work will indicate the crucial role played by RBPs in regulating important signalling cascades and go on to show that changing three bases in the 3' UTR of genes regulated by RBPs could show multiple developmental defects.

356A **Loss of mammalian glutaminase orthologs impairs sperm function in *C. elegans*** Qifei Liang, Haiyan Yang, Zhifei Zhang, Jialin C Zheng, Zhao QinTongji University

The decline in sperm function is a major cause of human male infertility. Glutaminase, a mitochondrial enzyme that catalyzes the hydrolysis of glutamine to generate glutamate, takes part in many diverse biological processes such as neurotransmission, metabolism, and cellular senescence. Here we report a role of glutaminase in regulating sperm function. By generating a triple mutant that harbors a loss-of-function allele for each of all three mammalian glutaminase orthologs, we found that glutaminase gene activity is required for optimal *C. elegans* sperm function. Tissue-specific gene manipulations showed that germline glutaminase activity plays an important role. Moreover, transcriptional profiling and antioxidant treatment suggested that glutaminase promotes sperm function by maintaining cellular redox homeostasis. As maintaining a low level of ROS is crucial to human sperm function, it is very likely that glutaminase plays a similar role in humans and therefore can be a potential target for treating human male infertility.

357A **Regulation of Developmental Arrest and Arousal at Hatching** Bruce Wightman, Long NguyenBiology, Muhlenberg College

Proceeding from embryogenesis to a free-swimming larva requires the coordinated execution of post-embryonic developmental program. Mutations in the neuronal differentiation transcription factors *fax-1* and *unc-42* cause a synthetic arrest phenotype at hatching, when combined with mutations in *daf-2/insulin receptor*, which we refer to as peri-hatching arrest. These results suggest that a neuronal function, perhaps a neuropeptide, potentiates insulin signaling to promote developmental progression at hatching. Arrested animals fail to initiate pharyngeal pumping and are largely inactive. Inactivity can be reversed by light stimulation and arrest can be rescued by mutations required for sleep, such as *flp-11*. Taken together, these observations indicate that peri-hatching arrest includes a sleep state. Peri-hatching arrest and inactivity can also be rescued by mutations in *ssu-1* or *nhr-1*, both of which are required for arrest in response to osmotic stress. We hypothesize that two opposed pathways, insulin and osmotic stress, coordinately regulate arousal and developmental progression.

We examined the role of the pro-sleep RIS neuron, which functions to promote sleep during larval lethargus. Mutations in *fax-1* did not affect RIS activity as measured using a GCaMP reporter. Late embryos that were arrested by 500mM NaCl or by *fax-1; daf-2* mutations displayed a decrease in RIS activity, indicating that the mechanism of the arrest sleep state does not involve stimulation of RIS activity. These observations suggest that the insulin-dependent step in progression and arousal is downstream of, or in parallel to, the pro-sleep function of the RIS neuron.

The pigment dispersing peptide of *Drosophila* plays an important role in regulating circadian clocks and the PDF-1 ortholog of *C. elegans* has a pro-arousal activity. We found that *pdf-1; daf-2* double-mutants arrest at hatching in an inactive state similar to *fax-1; daf-2* double-mutants. These observations suggest that PDF-1 also plays a stimulatory role at hatching.

358A **Manipulating malonyl-CoA entering the germline** Todd A Starich, David GreensteinGenetics, Cell Biology, and Development, University of Minnesota

Malonyl-CoA (mal-CoA) is produced by acetyl-CoA carboxylase (ACC, *C. elegans pod-2*). Mal-CoA is the rate-limiting substrate for fatty acid synthesis and a regulator of fatty acid oxidation; it is also implicated in regulation of energy homeostasis, neural stem/progenitor cell activity or quiescence, and AMPA receptor trafficking, among other processes (reviewed by Fadó et al., 2021). In addition, post-translational malonylation of lysine residues is a widely documented protein modification.

We showed that mal-CoA produced in the *C. elegans* somatic gonad is provided to the germline through gap junctions, minimally to support rescue of the Pod (polarity and osmotic defect) phenotype in fertilized embryos. As ACC is a target of the nutritional sensor AMPK, mal-CoA produced in the soma is ideally positioned to relay nutritional information to the germline. We have been exploring if mal-CoA production in the somatic gonad may influence earlier events in germ cell development.

We have used multiple approaches to manipulate mal-CoA levels, including AID tagging of *pod-2* and tissue-specific expression of malonyl-CoA decarboxylase (*mlcd-1*). Both approaches have been effective: AID-tagged POD-2 is most sensitive to TIr-1 -driven degradation in the hypodermis, while intestinal degradation is only effective at high IAA levels; tissue-specific expression of *gfp::mlcd-1* in the intestine arrests worms at the L2 stage, corroborating enzymatic breakdown of mal-CoA. However because of technical difficulties neither approach has yet been useful in targeting *pod-2* expression in the somatic sheath.

Most promising has been isolation of a strong regulatory mutant of *pod-2* resulting from imprecise excision of the SEC. This mutant has a novel phenotype– younger adults produce almost exclusively Pod embryos, while older adults produce mostly viable embryos. GFP expression is reduced in both the intestine and gonadal sheath compared to non-mutant strains. The proportion of Pod embryos laid can vary from ~40% to >85%. We conclude that high levels of mal-CoA production must be synced to the onset of meiotic maturation, and a delay in mal-CoA production accounts for the mutant phenotype. This phenotype can be rescued to wild-type by expression of a *lim-7p::gfp::pod-2* construct, consistent with our model that mal-CoA transits from somatic sheath to germline. We are now using this novel mutant to explore potential genetic interactions with gap junction hypomorphs.

359A **FBF directly represses *gld-1* RNA in germline stem cells and then activates it upon meiotic entry** Sarah L Crittenden[1], Chen Qiu[2], Jennifer Woodworth[1], Hezouwe Walada[1], Deep Kapadia[1], Traci M Tanaka Hall[2], Marvin Wickens[1], Judith Kimble[11]Biochemistry, University of Wisconsin - Madison, [2]Epigenetics and Stem Cell Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

PUF (for Pumilio and FBF) RNA binding proteins regulate stem cells across animal phylogeny. They bind with high affinity to defined elements and are best known as broad spectrum RNA repressors (1). Two nearly identical *C. elegans* PUF proteins, FBF-1 and FBF-2 (collectively FBF), regulate germline stem cells (GSCs), with the *gld-1* mRNA as a major target (2,3). FBF repression of *gld-1* expression is well established (2,3), but FBF activation was also proposed (4). However, evidence for activation was based on removing FBF genetically, an approach that does not exclude indirect effects.

To test whether FBF works directly to activate *gld-1* expression, we mutated FBEs with CRISPR/Cas9 gene editing. In the *gld-1* 3'UTR, FBF binds two canonical FBF binding elements (FBEs), called FBEa and FBEb, and one non-canonical element adjacent to FBEa, called FBEa*. We made FBEa, FBEa* and FBEb single mutants and FBEa FBEa* and FBEa FBEb double mutants in the endogenous *gld-1* gene. The three single mutants were fertile, but doubles made only sperm.

We next analyzed effects of FBE mutants on expression, using GLD-1 immunostaining for the *gld-1* endogenous gene. To circumvent germline masculinization of the double mutants, we also made FBE mutants in a *gld-1* 3'UTR GFP reporter (5) and analyzed effects on expression using GFP fluorescence. Remarkably, FBE effects were comparable in the two assays. Expression with a wild-type 3'UTR was low in GSCs and became high upon entry into meiotic prophase, as expected from previous work (5, 6). In the FBE double mutants, expression was higher than wild-type in GSCs, as expected for release from FBF-mediated repression. In addition, expression was lower than wild-type at meiotic entry, which we interpret as release from FBF-mediated activation. Major defects in FBF repression were seen in FBE single mutants, most notably FBEa, while major defects in FBF activation were only seen in the FBE double mutants, most notably FBEa FBEa*. We conclude that FBF acts directly to repress *gld-1* in GSCs via binding to a single FBE, and that FBF acts directly to activate *gld-1* in differentiating GSC daughters via synergistic binding to two FBEs.

1. Goldstrohm et al. (2018) TIG, 35, 972 **2.** Crittenden et al. (2002) *Nature*, *417*,660 **3.** Merritt et al. (2008) *Current Biology* **18**, 1476 **4.** Suh et al. (2009) *Genetics*,*181*,1249 **5.** Theil et al. (2019) *Nature communications* 10, 420. **6**. Brenner and Schedl (2016), Genetics 202, 1085.

360A **DAF-16/FOXO functions to both promote and oppose adult cell fate during dauer-interrupted development** Matthew J Wirick[1], Himani Galagali[2], Allison Cale[1], Benjamin S Olson[1], Isaac T Smith[1], Amelia F Alessi[2], John K Kim[2], Xantha Karp[11]Central Michigan University, [2]Johns Hopkins University

In some animals, development can be interrupted by a stress-resistant diapause stage to withstand adverse environmental conditions. The mechanisms by which developmental pathways are modulated to accommodate this interruption are poorly understood. One candidate for coordinating development and diapause is the FOXO family of transcription factors that regulate diapause across species. The sole FOXO ortholog in *C. elegans*, *daf-16,* promotes dauer diapause midway through larval development. We have shown that *daf-16* can also modulate multiple developmental pathways, including the regulation of stage-specific seam cell fate. We found that *daf-16(0)* dauers misexpressed the *col-19p::gfp* adult cell fate marker as well as 19/22 endogenous adult-enriched collagens, indicating that *daf-16* prevents precocious adoption of adult cell fate during dauer. Surprisingly, *daf-16* appears to play an opposite role after dauer: we found that expression of *col-19p::gfp* and other adult characteristics were reduced in *daf-16(0)* post-dauer adults. The regulation of adult cell fate is controlled by the heterochronic gene network, which has been studied primarily in non-dauer development. The LIN-29 transcription factor promotes adult cell fate most directly, including direct activation of *col-19* transcription. Early in larval development, translation of the *lin-29a* isoform is repressed by the LIN-41 RNA-binding protein. Late in larval development, *lin-41* expression is downregulated by the *let-7* microRNA. In *daf-16(0)* mutants, we found that *let-7* levels were upregulated during dauer and downregulated after dauer, consistent with the opposing *col-19p::gfp* phenotypes. As expected for a *let-7* target, we found that *lin-41* expression was reduced in dauer larvae. Furthermore, we showed that *lin-41* functions downstream of *daf-16* to oppose *col-19p::gfp* expression during dauer. Experiments addressing the role of *lin-41* after dauer are still ongoing. Interestingly, in contrast to the requirement for *lin-29* for *col-19* transcription during non-dauer development, we found that the misexpression of *col-19p::gfp* in *daf-16(0)* dauers occurs independently of *lin-29*. However, *lin-29* may play a role in the post-dauer *daf-16(0)* phenotype because expression of an endogenously tagged *lin-29::gfp* was reduced in *daf-16(0)* post-dauer L4 larvae. Together, we find opposing roles for *daf-16* in the regulation of seam cell fate during dauer development. In these contexts, *daf-16* appears to function partially through the known heterochronic gene network and partially via novel mechanisms.

361A ***Odd-skipped* Genes in *C. elegans*** Amy C. GrothBiology, Eastern Connecticut State University

The *odd-skipped* family of genes are transcription factors that are important for development. In mice and humans, Odd-Skipped-Related 1 and 2 are implicated in the development of various tissues (heart, lung, kidney, etc.) and cancers. There are two *odd* genes in *C. elegans*, *odd-1* and *odd-2.* Both are expressed in the intestine. Here we report that, in addition to intestinal expression and expression outside of the intestine in what may be the rectal gland cells, ODD2::GFP from an integrated array is also expressed in what appear to be sheath cells ~20% of the time. We have also identified a possible ODD binding site with similarity to *Drosophila* Odd and mouse OSR1 and OSR2 binding sites and we are currently testing candidate gene expression via q-RT-PCR in an *odd-1* deletion mutant strain compared to wildtype.

362A **Digital Integration of Neural Morphogenesis** Anthony Santella[1], Mark Moyle[2], Leighton Duncan[2], Nabor Vasquez Martinez[2], Ryan Christensen[3], Min Guo[4], Yicong Wu[4], William A Mohler[5], Daniel Colón-Ramos[2], Hari Shroff[3], Zhirong Bao[11]Sloan Kettering Cancer Center, [2]Yale University, [3]Janelia Research Campus, [4]National Institute of Biomedical Imaging and Bioengineering, [5]University of Connecticut Health Center

WormGUIDES is an integrated 4D atlas of neural morphogenesis, a data driven record of the emerging structure of the nervous system. The process of creating WormGUIDES has revealed much about nervous system structure and development. Insights gained include structural information about entry points, tissue scaffolds and stratified neurite organization within the nerve ring, as well as pioneering cell processes and novel mechanisms and strategies for collective polarization and extension neurons. In addition, the effort has revealed how collective tissue movement positions neurons prior to neurite outgrowth with a mechanism that resembles vertebrate neurulation.

WormGUIDES integrates neurite outgrowth dynamics from time lapse recordings within a carefully abstracted model of neural tracks and other anatomical structures. Tracts and the sorting of neurites within them are informed by the many sources of information integrated during the WormGUIDES project including fluorescence microscopy records of most embryonic neuronal outgrowth, and new analyses of electron micrographs.

This model and the custom software interface that houses it comprise a tool for both discovery and storytelling. It allows a user to move through time and space, exploring the model while adjusting the appearance of cells and other structures to highlight events of interest and observe spatial and temporal relationships. The interface also integrates diverse community resources, making it possible to quickly access existing knowledge to aid interpretation. As the user's understanding develops, they can bookmark and annotate areas of interest within a built-in content creation interface. The resulting 'story' packages custom an-

notation and appearance into a distributable interactive visualization that can be used for teamwork or illustration. We demonstrate the storytelling potential of WormGUIDES by using the application to explain several of the key biological insights that have emerged from our experiments.

363A **Characterizing the genetic and physical interaction of the DBL-1/BMP signaling pathway with BLMP-1/BLIMP1 transcription regulator in *Caenorhabditis elegans*** Mohammed Farhan Lakdawala, Tina L. GumiennyBiology, Texas Woman›s University

Animals use multiple signaling pathways for cell-to-cell communication for proper development. One signaling pathway is defined by its ligand family of bone morphogenetic proteins (BMP). In the roundworm *C. elegans*, BMP member DBL-1 has a well-defined, conserved pathway. The DBL-1 signaling pathway is involved in a spectrum of traits, including body size, brood size, and others. How does this BMP pathway control target gene expression? We are using *C. elegans* to address this question. Previous studies in *C. elegans* show that transcriptional regulator BLMP-1 affects a similar array of traits as DBL-1. However, the relationship between DBL-1 and BLMP-1 is not studied. We discovered that DBL-1 and DBL-1 signaling are affected by loss of BLMP-1. We also found that DBL-1 negatively regulates *blmp-1* expression. Additionally, ChIP-seq and RNA-seq data analyses suggest that DBL-1 pathway and BLMP-1 control expression of some common target genes, and act together.

364A **Ror1/CAM-1 receptor cooperates with Frizzled/MOM-5 receptor to control asymmetric division and gastrulation during *C. elegans* embryogenesis.** Takefumi Negishi, Sohei Nakayama, Mayumi Ohnami, Hitoshi SawaNational Institute of Genetics

Secreted proteins and their receptors are fundamental to patterning and morphogenesis in multicellular organisms. The Wnt pathway is involved in critical developmental events in many organisms. In most somatic cells of *C. elegans*, although Wnt signaling regulates asymmetric divisions, which show the stronger nuclear accumulation of TCF/POP-1, the transcription factor, in the anterior daughter cell than that in the posterior daughter cell, Wnts and their receptors regulating this POP-1 asymmetric accumulation (POP-1 polarity) in the mid-stage embryo have not been identified. The mutant of Frizzled/MOM-5, which is the only Wnt receptor essential for embryogenesis, shows normal asymmetric division. We found, however, that MOM-5 and Ror1/CAM-1 double-receptor deficient embryos failed to establish POP-1 polarity, showing that these Wnt receptors have redundant functions. We observed random orientation of POP-1 polarity in a double mutant lacking the Wnt-binding domain of MOM-5 (ΔCRD) and *cam-1* null mutation. Random POP-1 polarity was also observed in *wls/mig-14* mutant deficient in Wnt ligand secretion. These results support that CAM-1 functions in the mid-stage embryo in parallel to MOM-5, and Wnt-receptor interaction orients normal POP-1 polarity.

Next, we revisited the function of MOM-5 in gastrulation, which has been reported to act as a Wnt receptor for endoderm internalization. Unexpectedly, we observed that *mom-5(ΔCRD)* showed normal gastrulation. In contrast, double mutants between *mom-5(ΔCRD)* and null mutation of CAM-1 resulted in gastrulation defect. It suggests that, in gastrulation, CAM-1 compensates for the loss of MOM-5's Wnt binding activity. Our study suggests that CAM-1 supports the MOM-5 functions at different developmental events, and cooperation of both receptors underlies the robustness of *C. elegans* embryogenesis.

365A **Quantitative guiding of cell fates in dynamic signaling environments during *C. elegans* vulval development** Ismail Hajji[1], Miguel-Eduardo Sambrano-Lopez[1], Francis Corson[2], Eric D Siggia[3], Wolfgang Keil[11]UMR168 Physico-Chimie-Curie, Institut Curie, [2]UMR 8550 - Laboratoire de Physique Statistique, Ecole Normale Supérieure (ENS), [3]Center for Physics and Biology, The Rockefeller University

C. elegans vulval specification constitutes a long-standing and well-studied paradigm of the ubiquitously encountered dynamical signaling contexts underlying organogenesis during metazoan development. Cell fates are specified via two conserved signaling pathways: (i) a graded inductive Epidermal Growth Factor (EGF) signal emitted by an organizer cell called anchor cell and (ii) lateral Delta-Notch signaling between neighboring precursor cells. We have previously developed an intuitive "gene-free" modeling framework for *C. elegans* vulval cell-fate dynamics which quantitatively reproduces a large variety of genetic experiments, including the cell-fate frequencies of partially penetrant alleles (Corson & Siggia (2012,2017)). While the mathematical model for cell-fate acquisition of VPCs is remarkably successful in predicting terminal cell-fate phenotypes, its numerous dynamical implications for fate acquisition remain to be tested.

To do so, here we combine: (i) Parsimonious mathematical modeling of the underlying cell-fate acquisition dynamics and (ii) controlled perturbations of *in vivo* signaling dynamics using temperature-sensitive mutant alleles of the EGF/MAPK and Notch signaling pathways. We first applied time-independent perturbations by growing animals at various temperatures between the permissive and restrictive limits, assigning fate patterns corresponding to each perturbation and comparing them to the predicted patterns based on our model. We validated the predictions in each allele/temperature and also in series of allele/temperature combinations, including pathway epistasis effects. Secondly, we inversed our model and asked which perturbations

should we apply to direct precursor cells to certain fates outcomes? Our model predicts dramatic cell-frate changes based on time-dependent perturbations. We applied the predicted time-dependent perturbations by changing the growth temperature at various times during the competence period of the precursor cells, and compared again the experimental outcomes to the predicted ones, observing excellent agreement. This shows that we were able to dynamically guide cells to desired/predicted fates in a quantitative fashion, *in-vivo*. Our results reveal that the power of "gene-free" models for cell-fate dynamics extends beyond static external signals and that these models are able to quantitatively predict cell-fate choices in complex signaling dynamics *in-vivo*.

366A **Refining our understanding of male tail tip morphogenesis with a toolkit and tissue-specific RNA-seq** Karin H. A. Kiontke[1], Alyssa Woronik[2], R. Antonio Herrera[3], Porfirio Fernandez[1], Raza Mahmood[4], D. Adam Mason[5], David H. A. Fitch[11]Biology, New York University, [2]Biology, Sacred Heart University, [3]Biomedical Research, Baylor School, [4]Biology, New York University – Abu Dhabi, [5]Biology, Siena College

The 4 tail tip cells (hyp 8-11) of *C. elegans* undergo male-specific changes in L4, making a round instead of pointed tail tip. A main regulator for this Tail Tip Morphogenesis (TTM) is the transcription factor DMD-3 (Mason et al. 2008). We are developing a protein reporter toolkit to study the cellular events during TTM. This led to a refined description of TTM: DMD-3 expression in the tail tip starts in late L3. After the L3/L4 molt, tail tip cells dissociate from the cuticle, adherens junctions disassemble, but plasma membranes remain intact. Actin and myosin concentrate in the tail tip as it rounds up and shortens. The basement membrane (BM) is folded accordion-like. Later, extracellular matrix components are laid down at the end of the tail, and DMD-3 expression ceases. At the end of L4, hyp8-10 and hyp13 begin to migrate up to the anterior of the cloaca. hyp11 remains separate and dorsal of the BM throughout TTM. RNA-seq of laser-dissected early L4 tail tips identified 561 differentially expressed (DE) genes in wild-type (WT) males vs. *dmd-3(-)* males and hermaphrodites. To validate our data, we made transcriptional reporters for 40 DE genes. We found male tail tip-specific expression for 20 of these genes. Analysis of the RNA-seq data showed that: (1) The transcription profile of *dmd-3(-)* tail tips is similar to that of hermaphrodites. (2) Only 40 DE genes were also found in a whole-genome RNAi screen for defective TTM (Nelson et. al. 2011). (3) GO analysis of genes that are more highly expressed in WT male tail tips finds enrichment of terms that indicate high translational activity, and of terms that are consistent with cells undergoing morphogenesis. (4) GO terms enriched for genes with reduced expression in WT males are consistent with these genes being involved in cuticle maintenance. (5) Only 4 collagens are upregulated in WT male tail tips; two of these, BLI-1 and COL-20, are found in the cytoplasm of the tail tip cells. (6) Several genes in the chondroitin synthesis pathway are DE. Their reporters are expressed in male tail tips, and knockdown results in a TTM phenotype. We hypothesize that a chondroitin proteoglycan is secreted into the space between male tail tip and L4 cuticle. FBN-1 is a candidate for the core protein. These studies contribute to cell biological understanding of a morphogenetic process. We also study the regulation of TTM by DMD-3 and the dynamics of gene expression during TTM (presentations by Fernandez and Jallad et al.).

367A **Identifying Functional Interaction Motifs Within *C. elegans* Eggshell Vitelline Layer Proteins** Julián Prieto, Ysabella Alcaraz, Angie Wang, Elelta Sisay, Essi Logan, Athanasios Syntrivanis, Sara OlsonMolecular Biology, Pomona College

Metazoan oocytes are coated with a layer of secreted extracellular glycoproteins. 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 will ultimately form the outermost layer of the multilaminar eggshell. We previously characterized the first set of vitelline layer proteins in *C. elegans* – CBD-1, PERM-2, and PERM-4. We found that CBD-1 is central to the structure of the vitelline layer, and recruits two functionally-independent protein complexes that promote fertilization (the EGG proteins) and vitelline layer integrity (the PERM-2/4 proteins). To understand at a deeper mechanistic level how the vitelline layer proteins are recruited and interact with one another, we used CRISPR to delete specific domains within each protein. We found that a flexible loop in the N-terminal region of CBD-1 recruits PERM-2/4 to the vitelline layer, while chitin-binding domains in the C-terminal region are required to anchor CBD-1 to the oocyte surface via the EGG complex. For PERM-2 and PERM-4, a small predicted amyloidogenic domain within each protein is essential for their cross-recruitment, and for interaction with the CBD-1 scaffold. Interestingly, these amyloidogenic domains are reminiscent of those found in zona pellucida proteins of the mammalian egg coat, suggesting possible conservation of structural features among egg coat proteins across evolution. We also identified a number of additional proteins that interact with vitelline layer proteins through co-immunoprecipitation and mass spec analysis. Interestingly, these 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. These new uterine layer proteins fail to assemble onto the outer eggshell in the absence of vitelline layer proteins, further underscoring the intricate and hierarchical nature of nematode eggshell assembly.

368A **Patterning the apical extracellular matrix (aECM) during entrance and exit from dauer** Valeri J Thomson[1], Wendy

Fung[2,3], Ashlee Tan[2], Maxwell G Heiman[2,31]Biology, Bard College, [2]Boston Children's Hospital, [3]Harvard Medical School

The apical extracellular matrix (aECM) serves as an interface between the external and internal environments. Its structure is modified to meet tissue-specific needs, and can also be remodeled in response to environmental and developmental changes. For example, under stress conditions*, C. elegans* enter the dauer stage and its cuticle aECM undergoes extensive remodeling. When the stress is removed, the animals resume normal development and the cuticle is again re-modeled. Dauer-specific changes in cuticle structure thus provide an ideal system to investigate developmentally-regulated aECM patterning. We focused on changes in the cuticle aECM that overlays sense organs, in particular the formation of a dauer-specific aECM structure referred to as the "truss" in the anterior and posterior deirid sense organs (ADE and PDE, respectively). The truss was defined through electron microscopy studies as an apparatus that supports the ADE and PDE sensory cilia in dauer animals, but the molecular basis of this structure is not known. Previous lineage analysis studies suggested that the truss is produced by socket glial cells in these sense organs, the ADEso and PDEso respectively. We identified a collagen protein, COL-56, that is expressed in the ADEso and PDEso glia only as animals enter the dauer stage, and then turns off in these glial cells upon exiting dauer. This observation suggests that the ADEso and PDEso activate a dauer-specific transcriptional program that includes expression of the collagen COL-56 and results in formation of the dauer-specific aECM truss. Our findings provide a molecular entry point to determining how tissue-specific aECM structures are patterned in response to developmental cues.

369A **TIAM-1 regulates protrusive activity during dorsal intercalation through both GEF and N-terminal domains** Yuyun Zhu[1], Jeff Hardin[21]Genetics, University of Wisconsin-Madison, [2]Integrative Biology, University of Wisconsin-Madison

Mediolateral cell intercalation is a morphogenetic strategy that is used throughout animal development to reshape tissues. Dorsal intercalation in the *C. elegans* embryo involves the mediolateral intercalation of two rows of dorsal epidermal cells to create a single row that straddles the dorsal midline, and so is a simple model to study cell intercalation. Dorsal intercalation is heavily dependent on F-actin-rich protrusions polarized at the extending edges of intercalating cells. Protrusive activity during dorsal intercalation is mainly controlled by the *C. elegans* Rac and RhoG orthologs CED-10 and MIG-2, but how GTPases involved in intercalation are regulated has not been thoroughly investigated. Here, we establish a role for *C. elegans* TIAM-1, a Rac-specific GEF, in regulating protrusive activity during dorsal intercalation. TIAM-1 has an N-terminal EVH1-like domain, a central PDZ-like domain, and a C-terminal DH/PH GEF domain. In *tiam-1* mutants lacking GEF activity, dorsal epidermal cells generate fewer protrusions during intercalation, leading to significantly increased intercalation time. TIAM-1 functions in parallel to UNC-73, a previously identified GEF also involved in regulating protrusive activity during dorsal intercalation. Loss of both *tiam-1* and *unc-73* function enhances the defects shown in single mutants. We also identify a novel function of the N-terminal domains of TIAM-1 in inhibiting the formation of protrusions. Mutant embryos that produce an N-terminally truncated form of TIAM-1 with deletion of the PDZ domain are able to generate significantly more short, but non-polarized protrusions compared to wild-type embryos. This ectopic protrusion phenotype can be partially ameliorated by eliminating the GEF activity of TIAM-1 at the same time. We suggest that the PDZ domain of TIAM-1 autoinhibits its GEF activity, a model we are currently seeking to test using biochemical assays. We are also currently seeking to identify candidate components that act upstream of TIAM-1 to regulate dorsal intercalation. Taken together, our data establish a role for TIAM-1 in regulating protrusive activity during dorsal intercalation through both its GEF and N-terminal domains.

370A **Identifying genes required for ELT-7-mediated transdifferentiation through a large-scale genetic selection and computational analysis of whole-genome sequences** Tsung-Han Yeh, Pan-Young Jeong, Pradeep Joshi, Joel H RothmanMCD Biology, University of California, Santa Barbara

The mechanisms that allow fully differentiated cells to be reprogrammed into different cell types by transdifferentiation (Td) are not well understood. We found that forced ubiquitous expression of the ELT-7 GATA transcription factor, which functions at the terminal stages of gut differentiation, results in widespread expression of a gut marker in many or all somatic cells. This expression subsequently fades in most cells, with the exception of post-mitotic cells from four organs: the differentiated pharynx, the hermaphrodite uterus and spermatheca, and the male vas deferens. Cells in those organs undergo dramatic remodeling, showing the detailed ultrastructural characteristics of normal differentiated intestinal cells. Td can be promoted throughout development and in even in adults in the pharynx, which has been pumping food for several days.

To probe the mechanisms underlying this striking Td event, we devised a potent, high-throughput genetic selection strategy for mutants that are incapable of Td based on the fully penetrant developmental arrest provoked by ELT-7-mediated Td. Conditional expression of ELT-7 by the auxin-induced degradation system allowed us to identify 660 mutant lines from 12 million mutagenized worms that survive widespread ELT-7 expression. To circumvent mapping and gene cloning methods, we developed a computational bioinformatics pipeline that identifies candidate genes causally linked to ELT-7-mediated Td based on statistical analysis of whole genome sequences of all non-backcrossed mutants. From ~100,000 SNVs, we identified those that are most likely to be causally associated with the selected phenotype by three criteria: 1) mutational density within a gene segment, 2)

non-random distribution of mutations within a gene, and 3) percentage of mutations in conserved amino acids. We used SNVs identified by the Million Mutation Project as a control for comparison of the distribution of non-selected mutations. By ranking genes with the highest scores across these criteria, we identified a set of ~10 candidate genes required for Td, two of which were confirmed by complementation tests and rescue experiments. This included the gene for CDK-12, which has been implicated in repression of heterochromatin. As this method does not require genetic mapping, it could be applied to non-viable phenotypes or to model organisms that do not have sex or undergo genetic recombination. Further, with sufficient numbers of mutants, novel protein domains and key residues required for function can be revealed from such high-throughput studies.

371A **Characterizing gonad-enriched and sex-biased transcripts in *Caenorhabditis elegans*** Mary B. KroetzBiology, Bellarmine University

Previously, genetic screens have identified a handful of genes that, when their expression was abrogated, led to defects in the development of the somatic gonad in *C. elegans*. However, pleiotropic genes that are essential for viability are often difficult to isolate in genetic screens and thus might not have been isolated in these previous genetic screens. Therefore, to identify additional genes responsible for promoting gonadal development, cell-specific RNA-seq of the somatic gonadal primordium was employed. We identified several hundred gonad-enriched transcripts, which are transcripts enriched for expression in the developing gonad compared to that of the whole animal, and approximately 250 sex-biased transcripts, which are transcripts that are more highly expressed in the developing gonad of one biological sex compared to the other. We are currently characterizing the role of the gonad-enriched and sex-biased transcripts in gonadal development by focusing on the ~18% of gonad-enriched and sex-biased genes that are also known to be essential genes. Because deletion of these gene would lead to the death of the animal, we are conditionally removing the expression of the gene specifically from the somatic gonad. We have generated a strain of *C. elegans* that targets gonad-specific degradation of gfp-tagged proteins. To study the role of the gonad-enriched and sex-biased transcripts in gonadal development, students enrolled in a genetics course at Bellarmine University are fusing the gfp sequence to the endogenous locus of the gene of interest using CRISPR. We are currently in the second iteration of the genetics lab course. Students designed and generated the needed constructs for editing the genome via CRISPR, and screened and verified that the intended genomic edit was present. Students were successful at editing two of two genes in the first iteration of the course, and we are currently editing two additional genes. The strains expressing the gfp-protein fusions will then be crossed to the strain that targets gonad-specific degradation of gfp-tagged proteins to elucidate the role of these genes in the gonad. This research allows biology students to be exposed to authentic research in a class setting while helping to better understand the role of a suite of genes in gonadal development *in C. elegans*.

372A **SHC-3 regulates DAF-16 to promote survival in L1 arrest** Lesley T MacNeilBiochemistry and Biomedical Sciences, McMaster University

Shc proteins are adapter proteins that mediate phosphorylation-dependent protein-protein interactions function in many different signaling pathways. These proteins are characterized by the presence of two phosphotyrosine-binding domains, an N-terminal PTB and a C-terminal SH2. A major role of Shc proteins is to recruit signaling molecules to activated receptors at the cell membrane, putting them in proximity with other signaling molecules and promoting cellular signaling. Two Shc genes have been identified in *C. elegans*, *shc-1* and *shc-2*. We identified a third, previously unrecognized *C. elegans* Shc gene, *shc-3,* that functions at the cell membrane to promote oxidative stress response, pathogen resistance, and survival in L1 arrest. We show that although both *shc-1* and *shc-3* are required for long-term survival in L1 arrest, they do not act redundantly but rather play distinct roles in this process. SHC-3 regulates DAF-16 nuclear entry and exit in response to stress function and its function in mediating survival during L1 arrest is DAF-16-dependent.

373A **Characterization of a temperature-sensitive allele of *egg-3*** Ariel Strouse, Ashna Hoque, Amber KrauchunasUniversity of Delaware

Egg activation is the series of cellular events that transition a fertilized egg into a developing totipotent embryo. Egg activation events include the resumption and completion of meiosis, changes to the egg's outer coverings, and rearrangement of the actin cytoskeleton. EGG-3 is one of three pseudophosphatases that regulate egg activation in *C. elegans*. We are studying a temperature-sensitive allele of *egg-3* in order to more fully characterize the molecular function of EGG-3. The *egg-3(as40)* mutation is a single missense mutation that causes hermaphrodites to be marginally fertile at 16°C and sterile at 25°C. We find that stability and localization of this mutant form of EGG-3 is normal at both the permissive and non-permissive temperatures. We also find that localization of MBK-2 and CHS-1 are normal in the *egg-3(as40)* mutant, even though these proteins are unable to localize properly in the *egg-3* null. Surprisingly, we observed that progeny from *egg-3(as40)* animals develop slowly compared to wildtype. We are currently working to establish whether the developmental delay is solely due to maternal contributions of EGG-3 or if *egg-3* is expressed at later developmental timepoints. If the phenotype is due to maternal EGG-3 this suggests that defects at egg activation can have long term developmental consequences.

374A **Quantitative embryology: New approaches to the measurement of cell lineages** Gunalan Natesan, Eric J Deeds, Pavak K ShahUCLA

Cell lineages are a major input into the specification of tissue and cell identities during embryonic development and are increasingly easy to trace in *C. elegans* with 3D timelapse microscopy and automated lineage tracing software. We propose a novel generalized metric inspired by graph theory to compare phenotypic measurements aligned to cell lineages during embryonic development, allowing us to distill comparisons between cell lineage trees into intuitive distances. Our proposed metric, the Branch Distance, is simply the Euclidean distance between vectorized forms of any two cell lineages and can be easily computed for entire embryonic lineages across individuals or for any pair of sub-lineages within or between individual embryos. We applied the Branch Distance to a database of *C. elegans* embryonic lineages with a focus on identifying patterns in developmental timing. Use of this metric uncovered interesting batch effects in otherwise wild type populations of embryos as well as structure in the phenotypic consequences of perturbation by RNAi. By examining the pattern of Branch Distances between somatic lineages within wild type embryos we define a marker-free indicator of lineage identity based on lineage-specific patterns of cell cycle length and discover a novel influence of Notch signaling on the cell cycle.

375A **Deciphering the role of Syndecan in regulating the number of cellular projections in polarized cells** Anna Caridys Ramírez Suárez[1], Raphaël Dima[1], Marianne Bah Tahé[1], Yann Aghiles Chabi[1], Lise Rivollet[1], Claire Bénard[1,2]1Biological Sciences, Université du Québec à Montréal, Canada, [2]U Massachusetts Chan Medical School

Proper morphogenesis is critical for the development of highly specialized cells. Disturbances in cell morphology are intrinsically associated with defects in cell functions. Important progress has been made in deciphering the morphologic processes of outgrowth and guidance of cellular projections. However, the regulation of the appropriate number of cellular extensions remains unknown. Studies conducted by our group have revealed that the conserved heparan sulfate proteoglycan SDN-1/Syndecan is central in regulating the number of cell projections in the excretory canal cell. This cell is an excellent model for morphogenesis studies, and it shares developmental mechanisms with neurons. Our genetic analyses have further shown that *sdn-1* functions cell-autonomously, likely at its plasma membrane, and that it appears to cooperate with the guidance molecule UNC-6/Netrin and its receptors UNC-5/UNC5 and UNC-40/DCC, as well as the extracellular membrane receptor integrin, to control cell projection number. We are progressing towards testing whether the precise localization and dynamics of these molecular players depend on *sdn-1* function. We are progressing towards screening for intracellular interactors of SDN-1 using a TurboID approach. Finally, we will elucidate how a similar mechanism is at play in developing neurons. We expect that these studies will contribute to uncovering fundamental mechanisms underlying cell decisions about the proper number of projections during development. Given the remarkable evolutionary conservation of developmental mechanisms, we anticipate that these studies will contribute to research for understanding the bases underlying several developmental diseases in humans.

376A **TORC2 in Germline Development** Anke Kloock, E. Jane Albert Hubbard NYU Grossman School of Medicine

Stem cell populations should only grow and expand if nutrient conditions allow. For this purpose, signaling pathways are in place that relay information about the surrounding environmental or organismal nutrient conditions to the stem cells. One such nutrient-sensing pathway is regulated by activity of the Target of Rapamycin (TOR) kinase. This kinase forms two complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2). TORC1 has been researched extensively, and its role in nutrient regulation particularly by amino acids is well characterized. In *Caenorhabditis elegans*, previous work from our lab showed that upon low nutrient conditions, loss of TORC1 components or its downstream targets, the establishment of the germline stem cell pool is impaired (Korta et al., 2012 *Development*). By contrast, the role of TORC2 has been characterized for its roles in dauer entry, fat storage and body size, mitochondrial homeostasis and brood size, but not for its role in germline stem cell development.

To begin to investigate whether and how TORC2 is affecting the germline stem cell pool in response to nutrients, we are characterizing the role of the key TORC2 component, *rict-1*/Rictor. So far, we found that available hypomorphic mutants as well as new CRISPR/Cas9 *rict-1* null alleles we generated display a highly reduced germline stem cell pool. Unlike the germline-autonomous roles of TORC1 components, however, the germline defect caused by loss of *rict-1* can be rescued by expression of *rict-1* in the intestine. I am currently testing several hypotheses for how intestinal TORC2 activity regulates the size of the germline stem cell pool, and its possible connections to nutrient availability.

377A **Inhibition of mitotic cell cycling and activation of CHK-2 at meiotic entry are independently controlled by SCF-$^{PROM-1}$** Ariz Mohammad[1], Jian Chen[1], Verena Jantsch[2], Tim Schedl[1,1]Genetics, Washington University School of Medicine, [2]Chromosome Biology, Max Perutz Laboratories

The switch of stem/progenitor cells to entry into meiotic prophase is a key event in animal germ cell development. Three redundantly acting posttranscriptional pathways promote the switch, the GLD-1, the GLD-2 and the SCF^{PROM-1} pathways. SCF^{PROM-1} is

an E3 ubiquitin ligase complex that degrades substrate proteins at meiotic entry that are expressed in stem/progenitor cells, where PROM-1 is the F-box motif containing substrate specificity subunit. SCF^{PROM-1} controls meiotic entry by repressing mitotic cell cycling, at least in part by degradation of cyclin E (CYE-1) at meiotic entry, and activation of the CHK-2 kinase, a central regulator of meiotic entry that promotes homologous chromosome pairing, synapsis, and double strand break formation. Recently, we showed that activation of CHK-2 is indirect, by SCF^{PROM-1} degrading the phosphatase PPM-1.D which inhibits CHK-2 prior to meiotic entry through sequestration at the nuclear envelope. We tested the importance of ppm-1.D in mediating other SCF^{PROM-1} functions. We find that ppm-1.D has no role in ectopic mitotic cell cycling observed in gld-1 prom-1 null double mutants and has no role in degradation of stem/progenitor cell expressed proteins CYE-1, replication initiation factor SLD-2, kinetochore protein KNL-2, condensin subunit CAPG-1 and meiotic prophase and DNA damage check-point protein SGO-1 at meiotic entry. Thus, SCF^{PROM-1} independently regulates inhibition of mitotic cell cycling and activation of CHK-2. However, we found that downregulation of the cohesin loader WAPL-1 at meiotic entry is, at least in part, CHK-2 dependent, indicating an additional activity of the PPM-1.D arm of the SCF^{PROM-1} pathway. PROM-1 is nuclear localized, as are the above proteins downregulated at meiotic entry, suggesting that SCF^{PROM-1} activity may be limited to the nucleus. We found that cytoplasmic stem/progenitor cell expressed RNA binding proteins SPN-4 and MEX-3 are not downregulated by SCF^{PROM-1}, while translational repressor GLD-1 has this function. Accumulation of check-point kinase CHK-1 is also stem/progenitor cell restricted. Interestingly, neither the SCF^{PROM-1} nor the GLD-1 pathways function in downregulation of CHK-1 at meiotic entry.

378B **The neuropeptides FLPs of *Caenorhabditis elegans* are capable of responding to environmental cues and modulating larval development through hormonal signaling.** Masahiro Ono[1], Risako Une[2], Natsumi Kageyama[3], Riko Uegaki[3], Takashi Iwasaki[3], Tsuyoshi Kawano[1,41]The United Graduate School of Agriculture, Tottori University, [2]Graduate School of Sustainability Science Tottori University, [3]Tottori University, [4]Graduate School of Sustainability Science, Tottori University Masahiro Ono[1], Risako Une[2], Natsumi Kageyama[2], Riko Uegaki[2], Takashi Iwasaki[1,2], and **Tsuyoshi Kawano**[1,2,†1] Department of Bioresources Science, The United Graduate School of Agriculture; [2]Department of Agricultural Science, Graduate School of Sustainability Science, Tottori University, Tottori, Japan.

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FMRFamide peptides, a significant neuropeptide family for diverse physiological processes, are conserved in invertebrates. Free-living and parasitic nematodes also possess FMRFamide-like peptides (FLPs). Our research has demonstrated that multiple FLPs of *Caenorhabditis elegans* participate in the promotion or suppression of larval development by responding to environmental cues such as food and dauer pheromone. For instance, FLP-1 and FLP-2 modulate insulin-like signaling through their respective ligands, INS-35 and DAF-28, and thereby regulate larval development.[1,2)] While the receptors for FLP-1 and FLP-2 have not yet to be identified, we have found that an FLP can modulate larval development through TGF-β signaling, with DAF-7 being the relevant ligand. Moreover, our screen for *neuropeptide receptor* (*npr*) genes that potentially encode FLP receptors has revealed several genes relevant to larval development. Among these, the G protein-coupled receptor NPR-15 is involved in modulating larval development by regulating transcription, production, and subsequent secretion of DAF-7.[3)] While we have not yet identified the ligand for NPR-15, our molecular genetic analyses have shed light on the molecules that are relevant to modulating larval development, as described above.

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379B **The prion-like domain of IFET-1 is critical for localisation to P granules, but not to it's protein interaction network.** Madhu Sharma Sengupta, Peter Boag Biochemistry and Molecular Biology, Monash University

P granules are highly dynamic organelles that coordinate gene expression in germ cells. Among the many proteins that localise to P granules is a conserved translational regulatory complex that includes the eIF4E-binding protein IFET-1. The localisation of IFET-1 to perinuclear germ granules requires a C-terminal prion-like intrinsically disordered region (IDR). We identified IFET-1 interacting proteins by immunoprecipitation of GFP-IFET-1 in combination with mass spectrometry (IP-MS) and found the expected translational regulators CGH-1 (DEAD-box helicase CGH-1 (DDX6)) and the putative RNA-binding protein CAR-1. Surprisingly, IFET-1 was associated with only one of the five eIF4E isoforms, IFE-3, suggesting that IFET-1 may translationally repress specific mRNAs based on the 7-methylguanosine cap on the 5' on the mRNA. We also found a strong association between germ granules and PGL-1, but not with the other germ granule components, GLH-1 or PGL-3. Interestingly, GFP-IFET-1, without the prion-like IDR, strongly interacted with CGH-1, CAR-1, and PGL-1, indicating that germ granule location is not essential for these

interactions. In *life-1(0)* null mutants CGH-1, CAR-1, and PGL-1, germ granule localisation was significantly affected, whereas the non-interacting protein GLH-1 was localised normally. We hypothesise that the IDR of IFET-1 is critical for liquid-liquid phase separation (LLPS) that drives its P granule localisation but not its association with CGH-1, CAR-1, and PGL-1. The IFET-1 complex serves as an ideal model to understand how LLPS is involved in RNA-protein complex formation.

380B **Understanding the roles of sperm supplied SPE-11 and its novel oocyte partner, OOPS-1, in *C. elegans* egg activation** Ji Kent Kwah[1], Tatsuya Tsukamoto[2], Andy Golden[3], David Greenstein[2], Aimee Jaramillo-Lambert[11]Biological Sciences, University of Delaware, [2]2.Department of Genetics, Cell Biology, and Development, University of Minnesota, [3]3.National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

The cornerstone of sexual reproduction is fertilization, where one sperm and one oocyte fuse, triggering egg activation and embryonic development. In many animals, the sperm provides a haploid genome, a pair of centrioles for the first mitotic division, and the signal for egg activation. Mutants lacking these factors are known as paternal-effect embryonic lethal (PEL). In *C. elegans*, *spe-11* is the only strictly PEL gene. Oocytes fertilized by sperm lacking SPE-11 have defects in egg activation and the egg-to-embryo transition resulting in embryonic lethality (Hill et. al. 1989; Browning & Strome 1996). The mechanism of SPE-11 action in these events has been a mystery. We identified an interacting partner of SPE-11 that is expressed in the oocyte; *oops-1*(oocyte partner of SPE-11). We show that OOPS-1 is localized to the cortex of oocytes. Similar to *spe-11* mutants, we found that deletion mutants of *oops-1* are embryonic lethal. We imaged oocyte meiosis *in vivo* in wild-type, *spe-11(hc90)*, and *oops-1(tn1898* null*)* strains. As previously published (McNally & McNally 2005), in *spe-11(hc90)* mutant embryos, the chromosomes segregate at anaphase I and II but fail to form polar bodies. However, *oops-1* mutants show several different meiotic phenotypes with approximately half arresting at either meiosis I or II and the remaining mutant embryos displaying a *spe-11*-like phenotype. Like the *spe-11* mutants, polar body formation of the *oops-1* mutant is disrupted in the examples that were not arrested in meiosis I. As previous genetic results suggested that *spe-11(hc90)* is a hypomorphic allele (L'Hernault et. al. 1988), we also analyzed *spe-11(tn2059)* null mutants. *spe-11(tn2059)* had meiotic defects similar to *oops-1* null mutants. In addition to meiosis, we are investigating the role of SPE-11 and OOPS-1 in the formation of the eggshell, which is essential for prevention of polyspermy and embryonic development. Both *spe-11(hc90)* and *oops-1(tn1898)* mutants display similar defects in eggshell integrity. We found that *oops-1* mutants have defects in chitin formation similar to what Johnston et. al. 2010 showed for the *spe-11(hc90)* mutant. Analysis of strains harboring fluorescently labeled proteins involved in chitin layer formation in the eggshell (CBD-1 and CHS-1) and the egg-to-embryo transition (MBK-2), shows that the localization and expression of these proteins remain unchanged. Thus, the source of the eggshell defects when OOPS-1 and SPE-11 are compromised is still under investigation.

381B **Deciphering the Neuronal Pathways Involved in Long-term Social Behavior Across Development** Eshkar Nir, David Scher-Arazi, Shay SternFaculty of Biology, Technion

individuals within the same population may dynamically change their social behavior (inter-individual interactions) across developmental timescales. While neuronal processes that control social behavior at specific life stages were identified in many species, it is still largely unclear how individuals continuously change their social behavior across different stages of development. In addition, the molecular mechanisms and neuronal circuits that dynamically organize social behaviors across developmental timescales are also unknown. Our lab recently developed a novel imaging setup that allowed for the first time to study developmental patterns of behaviour of isolated *C. elegans* individuals at high spatiotemporal resolution and under tightly controlled environmental conditions. Here, we developed our imaging and computational methods for simultaneous monitoring of multiple pairs of *C. elegans* individuals from egg hatching to adulthood. Using this imaging system, we can characterize inter-individual behavioral interactions throughout the full generation time of animals. We found that wild-type animals show dynamic patterns of social interactions across development that may be different from pair to pair. Furthermore, we hypothesize that specific neuromodulatory circuits and sensory modalities function to establish differences in inter-individual behavioral interactions across different stages of development. We tested inter-individual interactions in mutants for specific neuromodulators, as well as genes involved in mechano- and chemo-sensation. Our results show that specific signaling pathways are involved in organizing the number of inter-individual behavioral interactions and interactions duration across development. These results highlight the importance of neuromodulatory and sensory pathways in organizing complex dynamics of social behavior across developmental timescales.

382B **Dissecting the Development of the *C. elegans* Pharyngeal Cuticle** Levon Tokmakjian[1], Muntasir Kamal[2], Jessica Knox[2], Duhyun Han[2], Houtan Moshiri[2], Lilia Magomedova[2], Ken CQ Nguyen[3], Hong Zheng[2], May Yeo[2], David H. Hall[3], Peter Mastrangelo[2], Jingxiu Ji[2], Hao Cai[4], Jakub W Wojciechowski[5], Michael P. Hughes[6], Kristóf Takács[7], Xiaoquan Chu[8], Jianfeng Pei[9], Vince Grolmusz[7], Malgorzata Kotulska[5], Carolyn L Cummins[2], Julie D. Forman-Kay[2], Peter J. Roy[21]Pharmacology, University of Toronto, [2]University of Toronto, [3]Albert Einstein College of Medicine, [4]The Hospital for Sick Children, [5]Wroclaw University of Science and Technology, [6]St. Jude Children's Research Hospital, [7]Eötvös University, [8]Peking University, [9]Tsinghua University

How the cuticles of the roughly 4.5 million species of ecdysozoan animals are constructed or constituted is not well understood. Here, we describe three approaches that we have taken in to better understand the construction and function of the *C. elegans* pharynx cuticle. In the first, we have systematically mined gene expression datasets to describe a spatiotemporal blueprint of cuticle construction. Using this approach, we demonstrate that various groups of intrinsically disordered proteins (IDPs) are incorporated via secretion and peak in expression in overlapping waves during cuticle construction. In the second approach, we have used fluorescently tagged and loss of function alleles for proteins predicted to be components of the pharyngeal to cuticle to better understand their localization within pharyngeal substructures. In the third approach, we have used a forward genetic screen and fluorescent probes to identify proteins required for the deposition of polar lipids into the pharyngeal cuticle. Overall, we demonstrate that IDPs play an important role in cuticle structure, whereas polar lipids safeguard the nematode from xenobiotics.

383B **NCAM-1 promotes synaptic remodeling in developing GABAergic neurons** Casey Gailey[1,2], Leah Flautt[1], Andrea Cuentas-Condori[1], John Tipps[3], Siqi Chen[3], Eleanor Rodgers[4], Seth Taylor[1], David Miller[1,2,51]Cell and Developmental Biology, Vanderbilt University, [2]Program in Developmental Biology, Vanderbilt University, [3]Vanderbilt University, [4]Saint Cecilia Academy, [5]Program in Neuroscience, Vanderbilt University

Neural circuits are actively restructured during development as synapses are dismantled in some locations and assembled in others. To investigate the underlying cell biological mechanism, we are exploiting the DD-type GABAergic motor neurons which undergo synaptic remodeling during early larval development. In the newly hatched larva, DD presynaptic boutons are initially positioned on ventral body muscles but are then relocated over a ~5 hr period to connect with dorsal muscles. The conserved homeodomain protein, IRX-1/Iroquois orchestrates DD remodeling. An IRX-1 target, the sodium epithelial channel (ENaC), UNC-8, is upregulated in remodeling DD neurons to trigger a Ca^{2+}-dependent mechanism of presynaptic disassembly. Additional downstream effectors are likely required, however, because UNC-8 dismantles a subset of presynaptic components (RAB-3, v-SNARE, liprin-a, endophilin) whereas IRX-1 also acts in a parallel pathway to remove additional presynaptic proteins (UNC-13, ELKS, Clarinet).

To identify additional remodeling genes, we used single cell RNA-Seq (scRNA-Seq) to profile D-class GABAergic neurons at periodic intervals spanning the remodeling period. Analysis of this data set revealed 93 genes that are transiently expressed in remodeling DD neurons. An RNAi screen detected a necessary role for the neural cell adhesion protein, NCAM-1, in DD synaptic remodeling. A genetic mutant of *ncam-1* impairs both the removal of ventral GFP::RAB-3 and its reassembly at dorsal DD neurites. Interestingly, the *ncam-1* mutant also delays remodeling of CLA-1/Clarinet, an active zone component that is not regulated by UNC-8. Thus, our results suggest that NCAM-1 functions in parallel to UNC-8 to promote DD presynaptic remodeling. Because NCAM functions as a key regulator of synaptic plasticity in mammalian neurons, we are intrigued with the possibility that NCAM-1 drives synaptic remodeling in *C. elegans* in a conserved mechanism that also governs circuit refinement in the developing brain. NIH Funding: 5T32HD007502 (CG), R01NS10695 (DMM).

384B **MRCK-1 activates non-muscle myosin to promote excretory canal development** Evelyn Popiel, Brent Derry Molecular Genetics, University of Toronto

The excretory canal cell of *C. elegans* provides a model system to interrogate the molecular mechanisms of biological tube development in the context of multicellularity. The growth and maintenance of this tube requires the dynamic coordination of the cytoskeleton and vesicle trafficking. Although many components of the cytoskeleton and vesicle trafficking required for tubulogenesis have been elucidated, our understanding of how these processes are regulated remains incomplete.

Previously our lab identified myotonic dystrophy-related Cdc42-binding kinase homolog 1 (*mrck-1*) in an RNAi screen for genes that regulate excretory canal development. Although the function of *mrck-1* and its homologs have been characterized in some cellular contexts, there is no known role for these kinases in biological tube development. My research aims to characterize the function of MRCK-1 in excretory canal development and define the pathway(s) it regulates.

Using CRISPR genome editing I investigated the requirement of key protein domains of MRCK-1 for its function in canal development. I showed that the kinase activity of MRCK-1 is essential for its function, while the Cdc42 and Rac1-interactive binding (CRIB) domain is dispensable. Using a cell-specific protein degradation system I discovered that MRCK-1 functions cell-autonomously in the excretory canal.

Given the canonical role of MRCK-1 as an activator of non-muscle myosin, I hypothesized that it also promotes the phosphorylation of the regulatory light chain MLC-4 in the excretory canal. To test this hypothesis, I introduced a phosphomimetic form of MLC-4 (MLC-4DD) in *mrck-1* mutants, which was able to rescue the canal truncations. Next, I investigated the requirement of MLC-4 in the excretory canal and found that, like *mrck-1*, it also functions in a cell-autonomous manner in the canal. Notably, the canal defects caused by loss of MRCK-1 and MLC-4 are very similar, which supports the hypothesis that *mrck-1* regulates *mlc-*

4 for excretory canal development.

My experimental findings support a model in which MRCK-1 activates non-muscle myosin to promote excretory canal development through phosphorylation of MLC-4. This provides the first example of *mrck-1* as a regulator of non-muscle myosin for tubulogenesis, which may be conserved in the development of biological tubes in a variety of organisms.

385B **Transcriptional analysis of *xol-1* mutant hermaphrodites reveals changes in developmental plasticity during embryogenesis** Eshna Jash, Anati Alyaa Azhar, Hector Mendoza, Halle Escher, Gyorgyi Csankovszki Molecular, Cellular and Developmental Biology, University of Michigan - Ann Arbor

xol-1, a gene located on the X chromosome, is the master regulator of sex determination and dosage compensation in *C. elegans*. *xol-1* is essential for normal development of XO males, where it promotes the expression of male-specific developmental genes. Additionally, *xol-1* inhibits genes important for hermaphrodite development, which include genes related to X chromosome dosage compensation. While *xol-1* is expressed at very low levels in hermaphrodite embryos, it is thought to be turned off at a very early stage, allowing male-specific developmental genes to be repressed. However, several aberrant phenotypes in *xol-1* mutant hermaphrodites suggest that it does have a function in hermaphrodites as well. We present evidence from bioinformatic analysis in wild-type and *xol-1* mutant embryos showing that the male-specific transcriptional program is active during early hermaphrodite embryogenesis. We also show that *xol-1* mutant embryos significantly overexpress *met-2*, which is a histone methyltransferase responsible for depositing the H3K9me2 mark. Overexpression of *met-2* and subsequent changes in H3K9me2 could potentially limit developmental plasticity in *xol-1* mutants. Consistent with that hypothesis, immunofluorescence experiments show that the dosage compensation complex, responsible for balancing gene products from the two X chromosomes in hermaphrodites, is activated earlier in embryogenesis in the absence of *xol-1*. These results suggest that XOL-1 may influence not only sexual differentiation but also the timing of the loss of developmental plasticity.

386B **The function of LEM-3 nuclease in regulating cell fate determination** Siyu Deng, Chaogu Zheng School of Biological Sciences, The University of Hong Kong

Cell fate determination is essential for the differentiation of multicellular organisms, especially the development of the nervous system that consists of many neuron types. The separation of cytoplasm during cell division allows the asymmetric distribution of cell fate determinants among the daughter cells, enabling them to differentiate into distinct cell fate. Using the mechanosensory touch receptor neurons (TRNs) in *C.elegans* as a model for cell fate specification, we identified a mutation that caused the formation of syncytium between the TRNs and their sister cells following the division of their precursor cells. The syncytium caused the labeling of the TRN sister cells by the fluorescent markers for TRN fate, suggesting a possible cell fate conversion. We mapped this mutation to *lem-3*, which is the *C.elegans* homolog of human ANKLE1 (Ankyrin repeat and LEM domain-containing protein 1) and contains a GIY-YIG endonuclease domain that is involved in resolving the holiday junction as well as in DNA damage response and DNA repair.

Based on the emergence of extra TRN-like cells in *lem-3(-)* mutants, we hypothesized that LEM-3 regulates cell fate determination by resolving the chromosomal linkage between sister cells to allow cytokinesis to occur to severe the cytoplasmic connection between the sister cells. The frequency of these unresolved chromosomal bridges is low, leading to a low penetrance of the phenotype in *lem-3(-)* mutants; however, various conditions that increase the frequency of DNA replication errors could increase the penetrance of the *lem-3(-)* phenotype. We further confirmed that the nuclease activity of LEM-3 is required for ensuring the separation of the TRN and their sister cells. The presence of nuclear envelope connection, in addition to the intercellular canal, also suggested chromosome linkage in *lem-3(-)* mutants; this unresolved chromosomal linkage likely resulted in the failure in cytokinesis in the last round of cell division that is supposed to generate two cells with distinct fates. We are in the process of understanding how LEM-3 nuclease coordinates the processing of DNA bridges and cytokinesis and safeguard cytoplasmic separation and the subsequent cellular differentiation. Besides, we will study whether LEM-3 has a general role in preventing syncytium and promoting fate specification in the nervous system by investigating the regulation of fate determination in broader types of cells.

387B **Identifying small molecule inhibitors of anchor cell invasion**
Wei Chen Kao[1], Simon Berger[1,2], Alex Hajnal[11]Molecular Life Sciences, University of Zurich, [2]Chemical and Bioengineering

During metastasis formation, invasive tumor cells breach basement membranes (BMs) and migrate across tissue boundaries to disseminate via blood vessels and form secondary tumors. Anchor cell (AC) invasion during *C. elegans* vulval development exhibits several similarities with malignant cancer cell invasion, as many of the genes regulating AC invasion are associated with metastatic tumor growth in humans. AC invasion therefore serves as a simple, accessible *in vivo* model to identify conserved regulators of cell invasion.

In order to find small molecule inhibitors of AC invasion, we screened 1280 compounds from the Library of Pharmacologically Active Compounds (LOPAC). Using a microfluidic-based screening platform developed in our lab (see accompanying abstract by Berger *et al*.), we imaged BM breaching in up to 50 drug-treated L3 to L4 larvae per compound and automatically scored AC invasion using a trained convolutional neural network. In this manner, we identified 13 compounds perturbing AC invasion. For example, exposing larvae to an inhibitor of the MEK kinase (UO126) strongly inhibited BM breaching.

Next, we will assay the activities of these compounds in different *C. elegans* mutant backgrounds to identify their targets and use cell invasion assays in other models, such as zebrafish larvae or mammalian tumor cell cultures, to test if the same signaling pathways control cell invasion in other species.

388B **Vitamin B12 metabolism controls MAPK-dependent cell fate decisions** Ana Laranjeira[1], Simon Berger[2,3], Tea Kohlbrenner[2], Alex Hajnal[21]Institute of Molecular Life Sciences, UZH, [2]Institute of Molecular Life Sciences, [3]Institute for Chemical and Bioengineering, ETH

Animal health depends on a balance between internal and external factors. Diet is an external factor that influences health by modulating nutritional availability and consequently metabolism. As such, physiological processes must adapt to changes in metabolism, which will affect signaling pathways on a cellular level. Here, we focus on vitamin B12-dependent metabolism, which is required for mitochondrial energy production, DNA synthesis and methylation. Specifically, B12 acts as an essential co-factor in the propionate breakdown pathway and the S-Adenosylmethionine (SAM) cycle. To study how vitamin B12-dependent metabolism affect cell fate decisions, we focused on different bacterial food sources (*E. coli* OP50 – low B12 diet – and *Comamonas aquosa* DA1877 – high B12 diet) and their influence on germ cell apoptosis, oocyte and vulval differentiation. We investigated the effects of these food sources on the RAS/MAPK signaling pathway, which transduces extracellular signals that regulate many physiological functions and is often hyper-activated in human cancer cells. In *C. elegans*, MAPK regulates vulval development, germ cell apoptosis and oocyte differentiation.

Worms fed with *Comamonas* displayed a 2- to 3-fold increase in germ cell apoptosis, increased oocyte numbers and enhanced vulval induction. This effect depends on the SAM cycle, since a loss-of-function mutation in the methionine synthase *metr-1* blocks the effects of a *Comamonas* diet. *metr-1(lf)* also suppresses phenotypes caused by MAPK pathway hyperactivation in the germline and vulva, indicating a relationship between the two pathways. Our results show that MAPK pathway activity is not directly regulated by B12 metabolism, but that downstream targets are up-regulated in B12-rich diet. Supplementing NGM with methionine, folinic acid or choline (three essential SAM cycle metabolites) enhances germline apoptosis and oocyte differentiation. Interestingly, vulval induction is only enhanced by choline or methionine, suggesting two distinct mechanisms may act in the germline and vulva. Finally, a *Comamonas* or choline-rich diet up-regulates histone H3K4 methylation, which promotes both vulva induction and oocyte differentiation.

Taken together, our results indicate that the diet influences essential physiological processes controlled by RAS/MAPK signaling. Given the strong conservation of the vitamin B12-dependent pathways, a similar metabolic regulation of RAS/MAPK signaling may exist in higher organisms.

389B **Deciphering transcriptional control of germ cell development** Qi Fan[1], Wei Cao[2], Roger Pocock[21]monash university, [2]Monash University

The *Caenorhabditis elegans* hermaphrodite germline is an efficient production line that produces ~300 progeny over 3 days. Each germline arm develops from a single primordial germ cell in L1 larvae to ~1200 germ cells in the adult. Germ cells proliferate in the distal end, maintained by GLP-1/Notch signalling. When displaced proximally, germ cells begin to differentiate and enter early meiotic prophase prior to gametogenesis and fertilization to produce progeny.

The regulatory network controlling germline development requires precise transcriptional regulation. The *C. elegans* genome encodes 875 transcription factors (TFs). Using germline-specific RNA-seq data, we detected 364 TFs that are expressed in the germline, with the majority having no ascribed germline function.

To discover TFs that are required for the development and function of the germ line, we performed RNAi and phenotypic analysis of germline development and germ cell behaviour. RNAi knockdown of individual TFs was conducted using a 'germline-specific RNAi strain' to investigate germline-autonomous roles of these TFs. Knockdown of 261 TFs caused perturbed germline development either at the germ cell proliferation or differentiation stage or both. We also identified 8 TFs that are essential for germline development as their knockdown caused sterility. In addition, SYGL-1 marker analysis revealed 54 germline-expressed TFs implicated in regulating GLP-1/Notch signalling in the distal germline.

Further analysis of the function of these TFs, we will gain understanding of transcriptional regulation in germline stem cell main-

tenance, cell fate specification, cell signalling and apoptosis.

390B **CMOS (Cellular Morphology of C. elegans Embryo), an online database for visualization and analysis of cellular morphologies and gene expression during C. elegans embryogenesis** Pohao Ye[1], Guoye Guan[2], Yiming Ma[1], Ming Kin Wong[1], Wing Sze Ho[1], Jianfeng Cao[3], Zelin Li[3], Zhongying Zhao[11]Hong Kong Baptist University, [2]Peking University, [3]City University of Hong Kong

The stereotyped development of C. elegans with invariant cell lineage permits systematic and unambiguous analyses of molecular and cellular control of embryogenesis with exceptional spatial and temporal resolution. To enable systematic and quantitative analysis of cell shape dynamics during embryogenesis, we develop a new segmentation method that allows segmentation of cell membranes up to 550-cell stage when most embryonic cells complete their last round of division with defined fate. To facilitate access to our data by the community, we present CMOS (Cellular Morphology of C. elegans Embryo), an online database for visualization and analysis of cellular morphologies and gene expression for all defined cells at 1.5-minute interval from 4-cell to 550-cell stage of C. elegans embryogenesis. Specifically, the database can allow integrative access to the reconstructed 2 dimensional (2D) and 3D cellular shapes, precomputed cell volumes, surface and contact areas that can be integrated with cellular expression of over 400 genes mainly consisting of transcription factors and signaling molecules. It provides customizable access to cell-cell contact network integrated with cellular expression profiles by query with a cell name or a specific time point. Interactive access to the qualitative and quantitative cellular data with high spatial resolution will facilitate the study of genetic and molecular control of C. elegans embryonic development.

391B **Systematic characterization of engulfment of apoptotic cells during *Caenorhabditis elegans* embryogenesis** Yiming MA[1], Dongying XIE[1], Pohao YE[1], Jianfeng CAO[2], Guoye GUAN[3], Zelin LI[2], Zhongying ZHAO[41]Biology, Hong King Baptist University, [2]City University of Hong Kong, [3]Peking University, [4]Hong King Baptist University

Apoptosis is an integral part of developmental process that ensures the elimination of unwanted cells at the right time and location during the development of multicellular organisms. *C. elegans* hermaphrodite embryo develops with an invariant cell lineage, during which 113 cells are programmed to die, and therefore is an ideal model for dissecting the control mechanisms of apoptosis.

Apoptotic cells in *C. elegans* are engulfed and eventually degraded by their neighboring cells through three partially redundant cell engulfment pathways, which are mainly deduced from the studies of a few post-embryonic apoptotic cells. Whether an apoptotic cell is randomly engulfed by one of its neighbors or by a specific neighbor remains elusive. Our lab has recently established a platform that allows automated reconstruction of cell surfaces with resolved cell identity up to 550-cell stage of a *C. elegans* developing embryo, which permits systematic identification of engulfing cell of apoptotic corpses. Aided by the platform, we are able to identify engulfment cells for a total of 58 apoptotic cells with 8 wild-type embryos. Although most of the apoptotic cells appear to be randomly engulfed by their neighbors, a subset of them are consistently engulfed by its specific neighbor, implying that distinct mechanisms are involved in the selection of an engulfing cell. We further demonstrate that a cell-specific engulfing cell usually maintains the largest contact area among all other neighbors since its apoptotic target is born, suggesting that the contact area is crucial for the assignment of engulfing cell. We also identify cells that perform sequential engulfment of multiple apoptotic cells, indicating the plasticity of engulfing capability of different cells during embryogenesis. To further dissect the mechanism of cell-specific engulfment, we generate a null mutant for a key apoptosis regulatory gene, *ced-3*, through CRISPR/Cas9. All of embryonic cells survive as expected, and engulfment behaviors appear to be altered. We are actively working on the engulfment behaviors and will report the results in the worm meeting. In summary, we perform the first comprehensive analyses of engulfment of apoptotic cells during *C. elegans* embryogenesis using reconstructed cell surfaces. It provides an entry point for the study of the molecular mechanism underlying cell-specific and non-cell-specific engulfment of apoptotic cells.

392B **Antagonizing Wnt signaling pathways converge on a small GTPase that regulates cell migration** Jonas D. Mars[1], Lorenzo Rella[2], Euclides E. Fernandes Povoa[1], Willemijn A. G. Bout[1], Rik C Korswagen[11]Korswagen lab, Hubrecht Institute, [2]School of Applied Biosciences and Chemistry, HAN University of Applied Sciences

The Wnt family of secreted glycoproteins regulates migration through distinct canonical and non-canonical signaling pathways. These pathways often antagonize each other, but the exact mechanism of this functional interplay is not known. In *Caenorhabditis elegans*, the QR neuroblast lineage provides a tractable model system to study this crosstalk. During the first larval stage of development, the long-range migration of the QR descendant QR.p is mediated through two parallel acting non-canonical Wnt signaling pathways, but once its daughter cell QR.pa reaches its final anterior position, migration is stopped through the activation of canonical Wnt signaling.

Previous work has shown that non-canonical Wnt signaling acts through the RhoGEF PIX-1 to stimulate the Rho-family GTPases that regulate migration. Furthermore, mRNA sequencing of isolated QR descendants revealed that activation of canonical Wnt signaling leads to upregulation of the target gene *rga-9b*, which is necessary and sufficient for termination of migration. *rga-9b* encodes a RhoGAP that may directly counteract the Rho-family GTPases that drive migration.

Here, we studied whether PIX-1/RhoGEF and RGA-9b/RhoGAP converge on the small GTPases MIG-2/RhoG or CED-10/Rac. We show that MIG-2 and CED-10 have distinct roles in regulating QR.p migration and we propose a model where CED-10/Rac is the nexus downstream of both PIX-1 and RGA-9b. Our model provides a link between canonical and non-canonical Wnt signaling and small GTPases to regulate cell migration via remodeling of the cytoskeleton.

393B **How is the number of natural transdifferentiations controlled in *C. elegans*?** jeanne cury, Thomas Daniele, sophie jarriaultIGBMC

Animals are composed of cells with various identities to form different cell types, tissues, and organs. We know today that cell identity, particularly the differentiated identity, can be changed: this phenomenon is called cellular plasticity. A special case is transdifferentiation (Td), which is a direct conversion from a differentiated cell type into another differentiated cell type.

The team uses the worm *Caenorhabditis elegans* as a model animal, which is particularly interesting to study Td *in vivo* thanks to its natural Td events during development but also its small cells number and its known invariable lineage.

In this worm, the rectal cell called "Y" transdifferentiates naturally into a neuron called "PDA" (Td Y-to-PDA), a robust phenomenon present in 100% of worms. Of the cells surrounding it, only the Y cell transdifferentiate: why only Y? The purpose of this project is to highlight mechanisms that restrict the number of cells that transdifferentiate.

We have previously highlighted a **positive selection** where the Y cell receives and interprets a **Notch signal**, allowing its Td. It is possible that the number of transdifferentiated cells is controlled by such positive selection mediated by Notch. However, at least another cell in Y vicinity can receive a Notch signal as well, and in situation with higher Notch activity, we found that this cell can transform into a competent Y cell that will transdifferentiate into a PDA neuron. Our preliminary data suggest that in the wild type, a negative selection mechanism might also be at play to restrict the number of cells able to respond to the Notch signal. We are now investigating the role of Notch antagonists in cells around Y to prevent their Td.

Overall, understanding such mechanisms will give insights on how cellular plasticity can be suppressed *in vivo*. The identified mechanisms could be further used to manipulate cellular identity *in vitro* for therapeutic purposes.

394B **A single nucleus perspective of pluripotent cells: investigating the genomic regulation of the M mesoblast lineage** Matthew A Hill[1], Julie A Ahringer[21]Gurdon Institute, Gurdon Institute, [2]Gurdon Institute, University of Cambridge

During embryogenesis, a totipotent zygote produces diverse cell types to form a functioning organism. Strict coordination of pluripotent stem cells give rise to the cells and tissues required for life. In *C. elegans*, the pluripotent M cell, born in the embryo, gives rise to the post-embryonic mesoderm during larval development. The cells of the M lineage are subject to diverse regulatory events including proliferation, two extended periods of quiescence, lineage specification, and differentiation into terminal cell fates making it an excellent model to understand how these different events are coordinated and specified. Through the work of many labs, several regulators of the M lineage have been identified, including conserved mesodermal master regulator HLH-8/Twist, pluripotency factor SEM-4/Sall, and chromatin regulators such as SWI/SNF (Harfe et al., 1998; Tian et al., 2011; Ruijtenberg and van den Heuvel, 2015). However, the genomic events and programmes that control entry into and exit from quiescence, the maintenance of pluripotency, lineage specification and differentiation are not well understood.

The invariant lineage of *C. elegans* allows for the examination and analysis of genomic regulatory events that govern developmental processes from mother to daughter cells. We have established the 10X genomics multiome platform in the Ahringer lab, which enables the simultaneous profiling of chromatin accessibility through ATAC-seq and transcription via nuclear RNA-seq from individual nuclei. By applying this to all descendants of the M cell, we aim to map the genome-wide chromatin activity at individual loci, together with the gene expression landscape.

This work will offer a comprehensive view of cellular development through the M lineage and enable the derivation of gene regulatory networks responsible for controlling cell proliferation, quiescence, and fate. Our results will advance our understanding of the molecular mechanisms underlying the regulation of cellular quiescence and differentiation, with potential implications for regenerative medicine and disease treatment.

395B **Collagen IV levels at the DTC/germline interface positively influences Notch receptor activation** Pier-Olivier Martel[1], Julia degrémont[2], Sarah Turmel-Couture[3], Eden Dologuele[4], Lucie Beaulieu[4], Patrick Narbonne[41]Universite du Quebec a

Trois-Rivieres, [2]Pathologie et Biologie Cellulaire, UQAM, [3]Biologie Médicale, UQTR, [4]Biologie médicale, UQTR

The basement membrane (BM) plays a crucial role in maintaining tissue structure and function and is implicated in stem cell regulation. In a forward genetic screen aimed at isolating mutations that promote GSC proliferation, we isolated a new allele of *mig-6L*, a gene coding for a protein homologous to mammalian Papilin, and which restricts the access of procollagen peptidase to the BM, thus negatively regulating collagen levels. This *mig-6L(qz2)* allele aggravates *glp-1(ar202)gf* conditional defects, increasing embryonic (+38.7%) and larval lethality (+9.1%), and germline stem cell (GSC) tumor formation (+380%) under permissive conditions. In addition, *mig-6L(qz2)* causes animals to develop shorter, but wider, distal tip cells (DTC) and distal gonads. Using a *Plag-2::GFP* (DTC); *EMB-9::mCherry* (COL IV) strain, COL IV being the most abundant structural BM component, we built 3D-models of distal *mig-6L(qz2)* gonads using confocal microscopy and Imaris. GFP expression from the *Plag-2* promoter was indistinguishable from control animals. We however discovered a significant increase in COL IV levels at the DTC/germline interface, specifically in the posterior gonad of *mig-6L(qz2)* mutants. We generated new 3D models, this time with animals having RFP-marked germ membranes and carrying a functional GLP-1(NICD)::GFP integrated transgene. GLP-1(NICD)::GFP expression levels were similar between control and *mig-6L(qz2)*. However, we found that Notch activity was higher in the posterior gonads of *mig-6L(qz2)* mutants based on the nucleocytoplasmic GLP-1(NICD)::GFP enrichment (*versus* membrane-localized). We then observed *daf-7* and *lon-2* TGF-beta pathway mutants, which both cause a general increase in COL IV levels. Each time DTC/germline COL IV levels were increased; Notch activity was also increased. The increased Notch activity observed in the presence of higher COL IV levels was confirmed by repeating assays with the Notch SALSA biosensor. Interestingly, DTC/germline COL IV levels were dramatically increased in GSC-less *glp-1(e2141)lf* mutants, while they were lower in tumorous *glp-1(ar202)gf* animals. A tumorous gonad having no notch receptor activity [*gld-3; nos-3; glp-1(lf)*] however showed control DTC/germline COL IV levels, suggesting that the increased COL IV levels are not due to the tumorous state of the germline. Overall, our results show a positive correlation between COL IV levels and Notch receptor activation, suggesting BM constitution may sizably influence intercellular ligand-receptor-mediated signaling. Our results also refute the paradigm that the increase in structural collagen levels that characterizes fibrosis is a consequence of elevated Notch activity.

396B **Investigating tissue-specific alternative isoform compatibility** Charlotte J Martin, John A CalarcoCell and Systems Biology, University of Toronto

Alternative splicing creates multiple mRNA variants from the same gene, influencing mRNA and protein stability, localization and protein interactions. Differences in splicing regulators across tissues can cause tissue-specific alternative splicing, creating distinct networks of transcripts between tissues, expanding the proteomic diversity of the organism. I am investigating the functional consequences of tissue-specific alternative splicing and its role in specializing gene function for different tissues. I have created a protein interaction network of genes that are alternatively spliced between neurons, muscle, and intestine in *Caenorhabditis elegans*. Next, I used CRISPR/Cas9 genome editing to generate single-isoform knock-in animals that exclusively express a single splice variant for several of the top interactors of this network. To date, I have successfully targeted the serine/threonine kinases AKT-1 and PKC-2, the endocytosis related proteins DYN-1 and DPY-23 and the cytoskeleton related protein VAB-10. I am currently exploring how exclusively expressing single tissue-specific isoforms affects animal fitness, as well as the effect it has on specific phenotypes such as lifespan, brood size, locomotion and behaviour. Previous studies investigating the functional consequences of alternative splicing often only look at single gene loci in isolation. However, I have created genetic crosses of these single-isoform knock-in animals to investigate how combinations of these tissue-specific isoforms affect the animal. Interestingly, I have found that animals expressing only the AKT-1 neuronal-specific isoform and PKC-2 intestine-specific isoform display a negative synthetic genetic interaction. Conversely, animals expressing both intestine-specific AKT-1 and PKC-2 isoforms have a significantly greater than expected relative fitness. My results suggest that there is an incompatibility of the neuron and intestine predominant isoforms of AKT-1 and PKC-2 when expressed in the same cells. The human orthologs of AKT-1 and PKC-2 are known to interact. To test if there are differences in the protein interactions of their tissue-specific isoforms I am also developing an *in vivo* tissue-specific single-copy bimolecular fluorescence complementation assay. My work will help elucidate how alternatively spliced genes work together and how disrupting these interactions could impact tissue function.

397B **Feminization of the Worm: An Exploration of the Role of *fem* Genes *in Caenorhabditis tropicalis*** Montana L.E.D. Bobinski[1], David Pilgrim[21]Department of Biological Sciences, University of Alberta, [2]University of Alberta

The Pilgrim laboratory has demonstrated that the species *Caenorhabditis elegans* is robust for studying the function and evolution of genes that regulate developmental decisions. In *C. elegans*, the number of sex (X) chromosomes determines the observable sex of the organism, either male (XO) or hermaphrodite (XX). However, mutations in key genes result in animals that ignore this chromosomal signal in sex determination. Utilizing the assumption that high protein sequence conservation will identify genes possessing similar developmental roles to those in *C. elegans* and *C. briggsae*, this project entails the use of a third closely related male/hermaphrodite *Caenorhabditis* species, *C. tropicalis*, which has a currently undefined sex determination pathway that provides an opportunity to identity the species' sex-determining genes and their functional and evolutionary relationships to

similar genes found in *C. elegans* and *C. briggsae*.

Using sequence comparison software to compare sex-determining gene sequences in *C. elegans* and *C. briggsae*, prospective sex-determining genes have been discovered in *C. tropicalis*. To induce knockout mutations in these *C. tropicalis* sex determining genes for functional analysis, CRISPR/Cas9 was adapted for novel use in *C. tropicalis*. Mutations have been generated in two of three identified *C. tropicalis fem* sex-determining genes, which have predictably produced feminized XX worms. Successful mutagenesis and analysis of the remaining *C. tropicalis* sex-determining genes and the deviations they produce in normal worm development, especially when compared against *C. elegans* and *C. briggsae* sex determination, will offer a greater understanding of the degree of evolutionary divergence and novelty possible in eukaryotic systems.

398B **Dissecting the role of PIE-1 in the specification of precursor germ cells during early embryogenesis in *C. elegans*** Pauline Ponsard[1], Sara Andus[2], Heesun Kim[3], Antonin Morillon[2], Craig C. Mello[3], Damien Hermand[11]URPhyM/GeMo, University of Namur, [2]UMR3244, Research University and Université Pierre et Marie Curie, [3]University of Massachusetts Medical School

In animals, germ cells are often specified or segregated from the somatic lineages during early embryogenesis. One feature that distinguishes precursor germ cells (PGCs) from somatic cells in all animals examined is the ability to produce new messenger RNAs (mRNAs) with the soma activating mRNA transcription earlier than PGCs. In *C. elegans* embryos, somatic blastomeres activate transcription at the 4-cell stage but mRNA expression is not detected in the germline blastomere until the 100-cell stage. This transcriptional repression is mediated by the maternally-expressed protein PIE-1, which becomes restricted exclusively to the germline blastomere from the first embryonic division. PIE-1 was proposed to stall transcription by inhibiting CDK-9, a Cyclin-Dependent Kinase (CDK) thought to be essential for Pol II CTD S2 phosphorylation and transcriptional elongation. However, the fact that a Pol II CTD S2A mutant completes embryogenesis and that CDK-12 is the genuine S2 kinase indicates that this model is either wrong or incomplete.

To explore the molecular basis of the repressive effect of PIE-1 on mRNA transcription, we have expressed PIE-1 in fission yeast where it blocks cell growth likely through transcription inhibition. Mass spectrometry after PIE-1 immunoprecipitation from arrested fission yeast cells has identified candidate targets, which we are analyzing.

To explore the function of PIE-1 in the C. elegans PGCs we are using PGC cell sorting combined with single-cell Multiome ATAC-seq / RNA-seq in the presence or absence of PIE-1. We will report on these datasets which we hope will allow us to better define the essential functions of PIE-1 in the maintenance of germline cell fate during embryogenesis.

399B **Developing methodology to identify regulators of early CEPsh glia development** Simin Liu[1], Georgia Rapti[2], Maria Nattestad[3], Shai Shaham[11]The Rockefeller University, [2]EMBL Heidelberg, [3]Google Health

Astrocytes are a major glial cell type in the vertebrate central nervous system, and play important roles in development, homeostasis, and plasticity of the brain. Surprisingly, unlike other glial cell types, specific genes and mechanisms that promote astrocyte cell fate determination remain largely unknown. *Caenorhabditis elegans* CEPsh glia may offer a model system to uncover elusive astrogliogenesis factors. These glia resemble astrocytes in their elaborate shapes, development from radial-glia-like precursors, postembryonic transcriptomes, and physiological functions. We have therefore sought to characterize the transcriptome of early CEPsh glia to identify genes that drive their formation. As with many *C. elegans* embryonic cells, specific reporters for isolating and characterizing early CEPsh glia have not been identified. To address this, we are using combinatorial gene expression approaches to identify reporters whose expression specifically overlaps in developing CEPsh glia. A reporter combination for the ventral CEPsh glia lineage has been identified, and using this reporter we isolated CEPsh glia, their progenitors, and their close relatives, and performed single-cell RNA sequencing to characterize their transcriptomes. Candidate developmental regulators identified from the transcriptome will be tested using CRISPR and RNAi knockdown methods for roles in CEPsh development. In addition, previous studies demonstrated that a *mir-228*p::gfp reporter is expressed early on in all *C. elegans* glia. We are bashing this promoter to identify regulatory elements specific for early CEPsh glia. We identified a small 64 bp region that largely retains pan-glial expression. This unexpected result raises the possibility of the existence of a pan-glial master regulatory element in addition to known glial-type-specific elements. Given the similarities between CEPsh glia and astrocytes, we are excited about the possibility that vertebrate homologs of CEPsh glia fate regulators we identify will also direct astroglia fate acquisition.

400B **SEM-2/SoxC regulates multiple aspects of *C. elegans* postembryonic mesoderm development** Marissa Baccas, Amy Leung, Vanathi Ganesan, Lucas Pineiro, Alexandra McKillop, Jun LiuMolecular Biology and Genetics, Cornell University

Development of multicellular organisms requires well-orchestrated interplay between cell-intrinsic transcription factors and cell-cell signaling. One set of highly conserved transcription factors that plays many different roles in development is the SoxC group. *C. elegans* contains a sole SoxC protein, SEM-2. SEM-2 is essential for embryonic development, and for specifying the

sex myoblast (SM) fate in the postembryonic mesoderm, the M lineage. We have identified a new partial loss-of-function *sem-2* allele, *jj152,* which has a proline to serine change in the C-terminal tail of the highly conserved DNA-binding domain. Detailed phenotypic analysis of the *jj152* mutation allowed us to uncover multiple new functions of SEM-2 in the M lineage. First, SEM-2 functions antagonistically with LET-381, the sole *C. elegans* FoxF forkhead transcription factor, to regulate dorsoventral patterning of the M lineage. Second, SEM-2 is essential for the proliferation and diversification of the SM lineage. Third, SEM-2 appears to directly regulate the expression of *hlh-8*, which encodes a basic helix-loop-helix, Twist transcription factor and plays critical roles in proper patterning of the M lineage. Our data, along with previous studies, suggest a possible evolutionarily conserved relationship between SoxC and Twist proteins. This study identified new interactions in the gene regulatory network (GRN) underlying *C. elegans* postembryonic development and adds to the general understanding of the structure-function relationship of SoxC proteins.

401B **DAF-12 mediates oleic acid dependent changes to metabolism and reproduction** Alexandra M Nichitean, Frances Compere, Sarah E HallBiology, Syracuse University

Starvation stress during key stages in early development can lead to phenotypic plasticity in adult animals. We use *Caenorhabditis elegans* dauer formation to investigate the mechanisms regulating starvation-induced environmental programming. When well-fed, L1 larvae develop continuously through the larval stages to become reproductive adults (controls, CON). However, if L1 larvae are faced with pending starvation, they may enter the stress-resistant dauer stage and resume development to become reproductive adults (postdauer, PD) when the dauer-inducing stress is alleviated. We have previously shown that *C. elegans* PD adults that experienced early-life starvation exhibited a downregulation of germline-expressed genes and upregulation of genes associated with fatty acid metabolism that correlated with lower fecundity and a decrease in stored intestinal lipids compared to controls. These phenotypes were dependent on the Δ9-desaturases, *fat-5, fat-6*, and *fat-7,* and the DAF-12 steroid signaling pathway. To elucidate the connection between metabolism and reproduction in PD adults, we performed brood size assays with PD hermaphrodites whose *E. coli* OP50 food was supplemented with different fatty acids. We found that oleic acid (OA) supplementation after dauer rescue increased brood size in a FAT-7 and DAF-12 dependent manner, and that similar increases in brood size were observed in CON animals supplemented after the larval L2 stages. Using a *fat-7::gfp* reporter and qRT-PCR, we showed that OA increases *fat-7* expression in a DAF-12 dependent manner. Furthermore, fatty acid profiling of PD and CON adults revealed that PDs have a significant increase in DGLA fatty acid, which can induce ferroptosis in the germ line. OA is known to block ferroptosis; thus, we tested if brood sizes of mutant strains in the ferroptosis pathway were altered with OA supplementation. Our results indicate that PD adults are more affected by ferroptosis, and that CON adults may have OA-dependent increases in fecundity by another mechanism. Moreover, we are currently identifying additional NHR proteins required for the OA-dependent phenotype. Our results suggest a model where DAF-12 has OA-dependent activity to regulate metabolism and fecundity and suggests that OA supplementation may ameliorate the negative consequences of nutritional programming in humans.

402B **Understanding the regulation and function of the CAP protein LON-1** Maria V Serrano[1], Lianzijun Wang[2], Zhiyu Liu[3], Anthony Nzessi[3], Myeongwoo Lee[2], Jun Liu[31]Cornell University, [2]Department of Biology, Baylor University, [3]Department of Molecular Biology and Genetics, Cornell University

Bone Morphogenetic Protein (BMP) signaling regulates a wide variety of processes in development and homeostasis, and its core pathway is conserved from humans to *C. elegans*. We are using *C. elegans* as a model to understand how BMP signaling is regulated and how BMP signaling regulates specific developmental processes. Mutations in LON-1, a protein in the CAP (Cysteine-Rich Secretory Protein, Antigen 5, and Pathogenesis-Related 1) superfamily, result in a long body phenotype and a mesoderm phenotype displayed by mutations in modulators of BMP signaling. Previous work has suggested that *lon-1* transcription is negatively regulated by BMP signaling (Morita et al 2002, Maduzia et al 2002). We have found that LON-1 also plays a role in regulating BMP signaling (Liu et al 2015). These findings led to a model that LON-1 both regulates BMP signaling and is regulated by BMP signaling, and that LON-1 may also have BMP-independent functions. We have identified a ~1kb region in the *lon-1* promoter that mediates BMP regulation of *lon-1* expression. This region is conserved in multiple nematode species and coincides with Smad binding sequences identified by ChIP-seq experiments. Mutating the putative Smad binding motifs in the 1kb promoter region rendered this promoter un-responsive to BMP regulation. These data together strongly suggest that *lon-1* transcription is regulated directly by BMP signaling. To dissect the mechanisms by which LON-1 modulates body size and regulates BMP signaling, we conducted structure-function analysis of LON-1 by determining the effects of point mutations at conserved residues of LON-1. We observed body size and mesoderm phenotypes in point mutants in putative sterol binding and ion binding sites in LON-1, suggesting that these activities may be important for LON-1 function. We have also generated functionally tagged LON-1 at the endogenous locus, and are using IP-MS to identify interaction partners of LON-1. Results from these experiments will provide insight into how LON-1 regulates body size and BMP signaling.

403B **Understanding the role of stop codon readthrough in *C. elegans* development** Loes B. Steller[1], Ludvík Hejl[2], Hidde

van Doorne[2], Suzan A. Ruijtenberg[21]Institute of Biodynamics and Biocomplexity, Utrecht University, [2]Utrecht University

Accurate cellular protein levels are crucial for cell function and can be achieved via precise regulation of transcription, translation and mRNA and protein turnover. Translational control is likely to be important during developmental transitions and cellular differentiation, which require dynamic changes in the proteome. We aim to elucidate the extent to which translational control, specifically stop codon readthrough (SCR), contributes to development and cell fate. Although SCR is often seen as a translation error, regulated SCR could increase protein diversity without any changes in transcription. Importantly, promoting SCR at premature stop codons in inherited diseases can rescue the disease phenotype. Indeed, multiple SCR-inducing drugs are currently tested in clinical trials. Stop codon recognition and translation termination are regulated by multiple factors, including eukaryotic release factors 1 and 3, tRNA levels, and mRNA sequence and structure. We aim to develop tools to study translation termination and get a better understanding of how SCR contributes to lineage specification in *C. elegans*.

We developed a dual-color reporter system that enables visualization of SCR in individual cells throughout development. Interestingly, using our reporter we already detected basal levels of SCR in a variety of somatic tissues. As expected, upon knockdown of release factors, we observed an increase in SCR in almost all cell types. To identify which additional factors - i.e., protein factors and/or (non-coding) RNAs - are responsible for accurate translation termination, we used our reporter strain to perform a pilot mutagenesis screen. This yielded six mutants that show increased levels of SCR, from which five mutants have a mutation in one of the release factors. This demonstrates that our reporter strain can be used to identify known regulators of translation termination, but most importantly also yet unidentified regulators of SCR.

To identify genes for which SCR is not an error, but an important and regulated event, we took a bioinformatic approach and integrated ribosome profiling data and sequence conservation analysis. Our approach identified several candidate genes with predicted SCR. Using our reporter, we aim to visualize these SCR events during development and identify the underlying mechanisms. Taken together, we developed tools that allow us to study how non-canonical translation events contribute to development and organismal behavior.

404B **Understanding how *kxd-1* contributes to homeostatic germline stem cell regulation** Armi Manharbhai Chaudhari[1], Patrick Narbonne[21]Department of medical biology, UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES, [2]UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

Stem cells can divide symmetrically to duplicate themselves or asymmetrically to produce one stem cell and a daughter cell that will differentiate. This fate decision is governed by niche signaling, while growth factors work largely in parallel to promote stem cell growth, and according to fate, their proliferation or differentiation. In *C. elegans* hermaphrodites, such growth factors activate the insulin/IGF-1 (DAF-2) pathway, which cell-autonomously promotes germline stem cell (GSC) proliferation downstream of nutrition, and the ERK/MAPK (MPK-1) pathway, which non-autonomously promotes GSC proliferation from the gut and/or somatic gonad. A homeostatic mechanism further suppresses GSC proliferation in sperm less animals as oocytes build-up in the proximal gonad arms. We used an *oma-1; oma-2* background to activate this homeostatic mechanism, and screened for mutants in which GSCs would keep proliferating despite a blockade in oocyte activation, and form easily detectable benign germline tumours. Using this strategy, we identified *kxd-1* as a new gene required for homeostatic signalling. Microarray and proteomics data suggest that KXD-1 physically interacts with several GTPase families, including ARL-8, as well as with G-protein signalling components GPR-1/2, and GOA-1. Interestingly, *goa-1* phenocopied *kxd-1* in the *oma-1; oma-2* background, suggesting that they may act together in the response to Major Sperm Proteins (MSP) that take place in the proximal sheath cells. We next performed *kxd-1*(RNAi) in a background that is only susceptible to RNAi in the germline (*rrf-1*) or in the soma (*ppw-1*). The *ppw-1* mutants were susceptible to *kxd-1(RNAi)*, whereas *rrf-1* mutants were resistant. We infer that *kxd-1* functions in somatic cells to prevent GSC proliferation in the absence of oocyte activation, consistent with its possible role in the response to MSP signals. We now aim to pinpoint the exact somatic tissue(s) where *kxd-1* function is required, and determine exactly its role in homeostatic germline regulation. A better mechanistic understanding of this new tumour suppressor role of *kxd-1* may lead to the development of new anti-cancer therapies.

405B **Identification of the tissue in which homeostatic signaling inhibits MPK-1 to block germline stem cell proliferation** Lloyd Venceslas FOTSO DZUNA[1], Patrick Narbonne[21]Medical Biology, Université du Québec à Trois-Rivières, [2]Université du Québec à Trois-Rivières

The Extracellular Regulated Kinase (ERK)/Mitogen-Activated Protein Kinase (MAPK) pathway is one of the major pathways for the transmission of proliferation signals. The transduction of these signals is coordinated by RAS, which upregulation is common in human cancers. We use the germline of the nematode *Caenorhabditis elegans* as a model to understand how ERK/MAPK controls stem cell (SC) proliferation, its genome encoding orthologs of RAS and ERK: LET-60 and MPK-1, respectively. Recent work has shown that the proliferation rate of SCs is coupled to the need for new differentiated cells in the tissue that these SCs

maintain. In *C. elegans*, germline SCs (GSCs) proliferate and their progeny differentiate to generate sperm in limited quantities, and then in adult hermaphrodites, oocytes that are fertilized by stored sperm. When sperm is exhausted however, unfertilized oocytes accumulate in the proximal gonad. This triggers a negative regulatory loop, which inhibits MPK-1 to block GSC proliferation. Although MPK-1 can stimulate GSCs proliferation from the gut or somatic gonad, the tissue(s) in which MPK-1 is inhibited to negatively regulate GSC proliferation remains to be determined. To identify this tissue, we expressed a *let-60* gain-of-function allele in a tissue-specific manner in spermless (*fog-1*) mutants to determine in which tissue MPK-1 overactivation would prevent homeostatic downregulation of GSCs. In parallel, we measured MPK-1 activity in the gut, muscle and sheath cells in wild-type and feminized (*fog-2*) animals. Our preliminary results suggest that MPK-1 must be inhibited in the gut, muscle and sheath cells in order for GSC proliferation to decrease in the absence of sperm. On the other hand, we found that MPK-1 is active in the gut and sheath cells of wild-type animals, but not in their body wall muscles. We are now in the process of measuring MPK-1 activity in these different tissues in *fog-2* mutants. In addition, using a labelled allele of MPK-1, we have observed that this protein migrates from the cytoplasm to the membrane in the oocytes of *fog-2* mutants, in a *daf-18* dependent manner. We will next determine in which tissue(s) DAF-18 is required for this change in subcellular localization of MPK-1.

406B **Specification of the *C. elegans excretory gland cell*** Marion E Boeglin[1], Oliver Hobert[1], Sophie Jarriault[2], Mayeesa Rahman[11]Columbia University, [2]IGBMC

A fascinating problem in neuroscience concerns the pathways that lead to the evolution of complex brains. At the base of this problem lies the question of how the first neuronal cell types evolved. One model posits that neurons evolved from highly secretory cells (Moroz, 2021). One specialized secretory cell type are gland cells. Are the genetic differentiation programs of gland cells homologous to those of neuronal cell types? We have set out to study this question in the nematode *C. elegans*, focusing on a specific gland cell type, the excretory gland cell. Lineally, the excretory gland cell is related to neuronal cell types. We are testing if the core regulatory principles of neuronal identity specification known to operate throughout the *C. elegans* nervous system apply to these specialized gland cells. Specifically, the goal is to determine whether excretory gland cell specification program break down, like neurons, into two parallel routines. (1) Terminal selector transcription factor(s) that coordinate control the distinct molecular identity features of the excretory gland cell, identified by single cell sequencing of the entire nematode (Taylor et al., 2021). (2) A parallel specification of the secretory machinery by the CUT homeobox genes as observed throughout the nervous system (Leyva-Díaz et Hobert 2022).

Neuronal terminal selectors are primarily encoded by homeobox genes (Hobert, 2021). The LIM homeobox gene *lim-6*, a terminal selector of various neuronal cell types (Gendrel et al., 2016) is expressed in the excretory gland cell (Hobert et al., 1999). We also find that *pros-1*, another candidate terminal selector of the homeobox gene family, is expressed in excretory gland cells. Preliminary genetic loss of function analysis indicates that both genes may act as terminal selectors of excretory gland cell fate. In regard to parallel regulation of the secretory apparatus, we find that the CUT homeobox gene *ceh-38*, indeed appears to control the expression of members of the secretory pathway in the excretory gland cell. Our preliminary data are consistent with the existence of similarities in the genetic architecture of the neuronal and gland cell differentiation, supporting the hypothesis of the ancestral relationship of these cell types.

407B **A sex-specific switch in a single glial cell creates a nanoscale pore in the extracellular matrix** Wendy Fung[1,2], Taralyn M Tan[1,2], Irina Kolotuev[3], Maxwell G Heiman[1,21]Harvard Medical School, [2]Boston Children's Hospital, [3]University of Lausanne

All epithelia are covered by an apical extracellular matrix (aECM) that provides a structural interface with the environment. Despite its critical role, how aECM is differentially patterned across tissues remains mysterious. To determine how aECM is patterned, we focused on the specialized cuticle aECM that overlies the endings of ciliated sensory neurons in the head. In particular, the cuticle overlying the CEP sense organs forms a closed sheet in hermaphrodites and juvenile males, but is remodeled in adult males to form a ~200 nm pore that provides the male-specific chemosensory neuron CEM access to the external environment. We discovered that a male-specific genetic switch in CEPso, a glial cell that wraps the CEM ending, is necessary and sufficient to create the cuticle pore. This switch is controlled cell-autonomously by the genetic sex of the glial cell itself, not by male-specific signals from neurons. Through candidate and unbiased genetic screens, we identified regulators of the sex-specific glial switch, including the sex identity factor *mab-3*/DMRT and repressor *nfya-1*/NFY-A. We found that MAB-3 acts in males to relieve NFYA-1-dependent repression of male gene expression in the CEPso glial cell. The switch results in male-specific glial expression of at least two aECM proteins: the Hedgehog-related protein, GRL-18 and a collagen, COL-53. Endogenously-tagged GRL-18 localizes transiently to nanoscale rings at the endings of sense organs where aECM cuticle pores will form, whereas endogenously-tagged COL-53 localizes stably to aECM structures that line the pores themselves. Using electron microscopy, we found that blocking male-specific gene expression in glia prevents pore formation, whereas forcing male-specific expression induces an ectopic pore in hermaphrodites. Together, our findings show that a switch in gene expression in a single cell is necessary and sufficient to remodel the aECM.

408B **Dafachronic acid-insensitive Daf-c enhancers in *Pristionchus pacificus*** Heather R. Carstensen, Sara Honardoost, Ray L.

HongBiology, California State University Northridge

The ability to form a dauer larval stage is shared among most nematodes as a stress-resistant dispersal stage capable of finding food and infecting hosts, yet the extent of conservation among genes that regulate and execute the dauer decision remain poorly described. The genetically tractable species *Pristionchus pacificus* is a useful model to identify potential novel genetic targets involved in dauer-specific developmental changes. Despite extensive reverse genetics targeting *daf* homologs, only mutations in the nuclear hormone receptor *Ppa-daf-12* and the transcription factor *Ppa-daf-16* appear to share the dauer defective (Daf-d) phenotype that is found in all nematode species investigated, while mutations in the *Ppa-daf-7* homologs and other members of the TGF-beta family do not exhibit any Daf phenotype. Although no mutations in the three *C. elegans hsd* genes result in a Daf phenotype, *Ppa-hsd-2,* the sole hydroxysteroid dehydrogenase in *P. pacificus,* is necessary for the biosynthesis of dafachronic acids and is the only characterized gene known to cause a constitutive dauer phenotype (Daf-c) in *P. pacificus*. The *Ppa-hsd-2* Daf-c phenotype can be rescued with exogenous Δ7-dafachronic acid (7DA), a functionally conserved hormone that suppresses dauer formation in various nematode species. To identify additional genes involved in dauer regulation in *P. pacificus*, we screened for genetic enhancers of *Ppa-hsd-2* and isolated nine "uber-dauer" Daf-c alleles that form dauers on 7DA. Three alleles also have a strong amphid neuron dye-filling defect (Dyf). While *Ppa-hsd-2* shows reduced nictation behavior compared to wild type, two uber-dauer alleles exhibit recovery of nictation behavior. With the range of phenotypes observed, we estimate that these nine uber-dauer alleles represent at least four affected genes. Whole genome sequencing revealed a *Ppa-din-1* homolog as a candidate in two of the enhancer alleles. Given the spectrum in the severity of Daf-c formation and the degree of recovery of dauer-specific nictation behavior in these enhancers, identification of the responsible genes may uncover several dauer regulatory genes not previously known to be involved in dauer formation in *C. elegans*.

409B **Mechanisms Mediating FBF Clearance within the *C. elegans* germline** Gabriella Weiss, Ekaterina VoroninaUniversity of Montana

Post-transcriptional gene regulation in *C. elegans* germline stem cells relies on RNA-binding proteins (RBPs) which are essential to stem cell self-renewal and differentiation (Hubbard & Schedl., 2019; Kershner et al., 2013). The FBF PUF-family RNA-binding proteins, FBF-1 and FBF-2, are essential for *C. elegans* germline stem cell maintenance (Crittenden et al., 2002). The FBF's are translational repressors expressed in germline stem and progenitor cells (SPCs) that use distinct mechanisms to silence mRNAs (Voronina et al., 2012; Wang et al., 2020). However, the regulation of FBF protein stability including the signal that triggers FBF clearance at the onset of meiotic differentiation is unknown. In the initial structure-function analysis, we identified an FBF-2 peptide that was sufficient to induce degradation when fused to GFP. We found that downregulation of this reporter was dependent on the proteasome activity. Through ongoing analysis, we aim to investigate the ubiquitin proteasome system with the goal of determining the E3 ligase responsible for the degradation of the GFP reporter and ultimately FBFs at the onset of meiotic differentiation. Our preliminary results identified five monomeric RING finger proteins downregulating the GFP reporter, which will be investigated further. So far, LIN-41 and MNAT-1 appear to be indirectly limiting FBF accumulation in the germline.

410B **Filopodia-mediated morphogenic cytokinesis during chiral morphogenesis** YuXuan Xiong, Gaganpreet Sangha, Kenji SugiokaUniversity of British Columbia

Chirality is one of the fundamental molecular and cellular properties that controls animal morphogenesis. In organisms with bilateral symmetries, molecular and cellular chirality specify the larger scale organismal handedness, wherein organ's relative positioning and shape exhibit left-right asymmetry. In *Caenorhabditis elegans*, body handedness is first specified during 4-cell stage cytokinesis in a CYK-1/Formin-dependent manner (Wood B., 1991; Middelkoop *et al.*, 2021). However, the mechanism underlying cell division-dependent chiral morphogenesis remains elusive. Here, we found that the contractile ring tilting and filopodia-dependent ring-cell surface coupling underly chiral morphogenesis in *C. elegans*. Specification of *C. elegans* organismal handedness is accompanied by clockwise rotation of ABa and ABp division axes along anterior-posterior axis at 4-cell stage cytokinesis. To identify the intrinsic and extrinsic cues that regulate chiral morphogenesis, we performed the blastomere isolation assay to eliminate and reconstitute cell-cell interactions. We found that adhesion between ABa and ABp is sufficient for chiral morphogenesis and that the attachment of adhesive polystyrene bead to either ABa or ABp induces the clockwise cell rotation. Consistently, the contractile rings of ABa and ABp in intact embryos undergo clockwise rotation relative to the ABa-ABp boundary, resulting in non-parallel alignment of the two rings. Furthermore, we found the Formin-dependent formation of interlocking filopodia at the ABa-ABp boundary. Interestingly, this newly found mitosis-specific filopodia extending from ABp appears to be pulled by the ABa contractile ring and vice versa. Because of the non-parallel ring alignment, it is likely that ABa and ABp contractile rings pull ABp and ABa cell surfaces toward the anterior right and posterior left, respectively, leading to asymmetrical boundary deformation and chiral blastomere arrangement. Our results illuminate the novel contractile ring-dependent morphogenesis mechanism critical for shaping the embryonic body plan.

411B **Optimization of neuron-specific isolation of nuclei for molecular profiling in *C. elegans*** Guihua Zhou, Dalton

Patterson, Haosheng SunUniversity of Alabama at Birmingham

Understanding cell-specific gene expression and function during development requires isolation of individual cell types for analysis. INTACT (Isolation of Nuclei TAgged in specific Cell Type) has been broadly used in model organisms from *C. elegans* to mice for the purpose of nuclei isolation from specific cell types for downstream molecular profiling. However, the isolation of rare cell types (i.e., single neurons) using this method can suffer from relatively high noise to signal. Here, we are optimizing methods for isolation of neuronal-specific nuclei and downstream molecular profiling. First, we compared fluorescence-activated nuclei sorting (FANS) to affinity purification using immunoprecipitation (IP), both in terms of efficiency of isolation and signal to noise ratio of downstream transcriptome profiling. Second, we optimized methods of reproducible and efficient nuclei isolation using machine-based protocol, rather than laborious manual isolation. Third, we are optimizing our methods of nuclei isolation to be compatible with snRNA-seq platforms. Lastly, we are optimizing chromatin profiling methods (ATAC-seq etc) with nuclei obtained from our isolation method. Together, we are optimizing methods to efficiently isolate single neuron nuclei for downstream molecular profiling that maximizes signal to noise ratio, so we may better understand the molecular composition of the nervous system in detailed resolution across conditions.

412B ***vab-6* encodes a kinesin and promotes epidermal morphogenesis through miRNA regulation** Dan C Quesnelle, Ian D Chin-SangBiology, Queen›s University

Morphogenesis, an organism's ability to generate proper body shape, is a crucial step in its development. 50 years ago genetic screens in *Caenorhabditis elegans* identified a group of mutants called ***v**ariable **ab**normal*, which exhibit morphological deformities that severely affect their growth and survival. While several *vab* genes have since been characterized, the identities of many remain unknown. We show that the *vab*-6 gene is synonymous with the *klp*-20 gene in *C. elegans*. KLP-20 encodes a kinesin motor protein and is an ortholog of the human KIF3A. KLP-20/KIF3A is a component of the kinesin-II motor complex involved in ciliogenesis. A single amino-acid substitution in the KLP-20 motor domain causes bumpy epidermal phenotypes and a range of defects affecting different aspects of their development. Curiously, a complete knockout of the *klp-20* gene does not produce these epidermal defects, suggesting that the disruption of the catalytic regions may produce an allele with neomorphic-like properties. Despite showing bumpy epidermal phenotypes, KLP-20 is expressed exclusively in the nervous system, suggesting that KLP-20 may be acting cell non-autonomously to regulate epidermal morphogenesis.

KLP-20 directly interacts with a component of the RNA-induced silencing complex, VIG-1, and therefore we hypothesized that KLP-20 transports miRNAs and miRNA machinery to the sites of translation of their target transcripts. *let-7* is a well-studied miRNA with well characterized targets in both *C. elegans* and mammalian models. In *klp-20*mutant animals, we observed an increase in expression of two of *let-7*'s targets, *lin-41* and *hbl-1*. Additionally, genetic knockdown of *let-7* in a *klp-20* mutant background resulted in increasingly severe bumpy phenotypes, strongly suggesting that *klp-20* is regulating *let-7* to some degree. MicroRNAs are rapidly being identified as post-transcriptional regulators in a wide array of processes and thus understanding the role of motor proteins in this layer of development is critical for our understanding of how gene expression is regulated during development as a whole.

413B **Notch is required for the completion of the late-developing avm branch at the *C. elegans* nerve ring** Rachid El BejjaniBiology, Davidson College

Most studies to date have examined axon growth and guidance during early development but less is known about these processes during later stages. The conserved Notch signaling pathway regulates key developmental processes and remains active after development is completed. We show that Notch is required for the growth of an axonal branch that connects the anterior touch neurons in *C. elegans* in the last larval stage, after most other neurons have completed their development. The late-developing AVM mechanosensory branch exhibits defects in all Notch pathway mutants tested. Data from repeated imaging and from independent experiments suggest that the defect is a failure in branch formation during late development and not a result of degeneration of a previously formed branch or of developmental delay. Tissue specific rescue experiments suggest that Notch functions non-autonomously, in tissues other than the adjacent body wall muscles. Interestingly, initial ventral guidance of the AVM neuron is not affected in Notch mutants, suggesting that Notch specifically affects the formation of the late, anterior branch.

414B **Investigating the Effects of COMT Loss in *C. elegans*** Salma AshShareef, Brian T NelmsFisk University

Dopamine (DA) is a crucial neurotransmitter regulating nervous system function including cognition and motor control. Dysregulation of DA signaling is associated with diseases like Parkinson's and schizophrenia. DA levels can be regulated at multiple steps in the pathway such as biosynthesis, packaging, release, reuptake, and breakdown(metabolism). Using C. elegans, we to investigate one of the main classes of enzymes that metabolize DA, which are the Catechol-O-methyl Transferases (COMTs). This

project aims to understand the impact of the loss of COMT-2 or COMT-4 on DA metabolism and worm function. To determine this, we are using assays for various behaviors that are impacted by DA levels such as Swimming Induced Paralysis, basal slowing, and egg-laying rate. Due to the conservation between human and C. elegans DA signaling pathways, our results may give important insight into regulation of DA levels, DA metabolism, and its corresponding therapeutic implications.

415B **The transcriptional landscape of nervous system development in both sexes of *C. elegans*** Rizwanul Haque[1], Sonu Peedikayil-Kurien[1], Hagar Setty[1], Yehuda Salzberg[1], Gil Stelzer[2], Einav Litvak[1], Hila Gingold[3], Oded Rechavi[3], Meital Oren-Suissa[11]Brain Sciences, Weizmann Institute of Science, [2]Bioinformatics Unit- Life Science Core Facilities, Weizmann Institute of Science, [3]Neurobiology, Tel Aviv University

In the animal kingdom, the nervous systems of male and female species have evolved distinct properties to facilitate sex-specific behaviors. However, the genetic mechanisms responsible for introducing these sex-specific features into the nervous system are not well understood. To address this question, we aimed to develop a comprehensive gene expression atlas for both sexes of *C. elegans* across developmental stages. Since distinguishing male and hermaphrodite larvae is difficult at early developmental stages, we developed a method to isolate large numbers of healthy males with high purity at an early stage. This enabled us to conduct whole-animal RNA-seq studies for both sexes across development for the first time. Our findings revealed a set of genes that are differentially expressed across developmental stages, including neuronally-expressed genes such as transcription factors, neuropeptides, and GPCRs. We validated our results using CRISPR transcriptional GFP knock-in strains and identified the insulin-like neuropeptide INS-39 as the top male-biased gene, highly upregulated in males at all developmental stages. Using the multicolor transgene NeuroPAL to identify cell types, we discovered that INS-39 is expressed specifically in ciliated sensory neurons with distinct functions, two in hermaphrodites and five in males. By masculinizing or feminizing the nervous system, we observed that the master sex determination regulator, TRA-1, functions to restrict *ins-39* expression in hermaphrodites, with a TRA-1 binding site identified in the *ins-39* first exon, but other factors are needed for male-specific expression. Additionally, the DMD gene *dmd-9* regulates INS-39 expression in both sexes. Finally, we used INS-39 CRISPR knock-out animals to evaluate its behavioral roles in the two sexes. We found that while *ins-39* regulates cold-tolerance in both sexes, it has a dimorphic and pronounced role in L1 survival in males. Taken together, our results show lower expression of INS-39 in hermaphrodites is fine-tuned to respond critically to environmental changes, while higher expression in males might act as a molecular impediment for nuanced signaling. Our study offers a comprehensive database of dimorphic gene expression across development and provides a basis for exploring the roles of individual genes in dimorphic development. Furthermore, it also highlights conserved candidate genes that may underlie the sexually-dimorphic manifestation of different human diseases.

416B **The Role of HPK-1 and HSF-1 in Linker Cell Death** Betty Ortiz[1], Lena Kutscher[2], Shai Shaham[11]The Rockefeller University, [2]Universität Heidelberg

To achieve proper development, a subset of generated cells are destined to undergo cell death. Insufficient or overabundant cell death can promote disease states such as cancer or neurodegeneration, respectively. Apoptotic cell death, whose molecular regulators were first identified in *C. elegans*, is characterized by chromatin condensation and cell shrinkage. This highly-studied cell death mechanism requires caspase proteases and other regulators. Surprisingly, mice in which this pathway is blocked can develop normally despite the prevalence of cell death in development, suggesting that other cell death mechanisms must play important roles. One such form of cell death is linker cell-type death (LCD), which is genetically and morphologically distinct from apoptosis. LCD is characterized by nuclear crenellations and swelling of cytoplasmic organelles, without chromatin compaction. LCD is regulated by multiple activating pathways: two antagonistic Wnt signals (LIN-44 and EGL-20); the transcription factor LIN-29; TIR-1, MAPKK SEK-1, and polyQ protein PQN-41; and the hormone receptor NHR-67. These pathways all converge to regulate the heat shock factor HSF-1. HSF-1 is well known for its pro-survival roles in response to heat and other stressors; however, during LCD it has an opposite function promoting cell death. Genetic studies suggest that in the linker cell, the pro-survival and death-promoting functions are derived from a common pool of HSF-1 proteins. From a 2-hybrid screen we identified the HPK-1 homeodomain-interacting protein kinase as an interactor with PQN-41. In other animals, homologous kinases phosphorylate HSF-1 and regulate the heat-shock response. Our preliminary studies suggest *hpk-1* mutants exhibit LCD acceleration and can suppress ectopic survival in *egl-20* mutants. Furthermore, we showed that an HPK-1::GFP reporter generated by CRISPR insertion into the endogenous locus is expressed in the linker cell, among other cells. We are examining the phenotypes of additional *hpk-1* mutants we designed, and aim to test how phosphorylation of HSF-1 by HPK-1 might affect the balance between cell death and survival.

417C **Cell-cell fusion and its regulation during *C. elegans* male tail morphogenesis** Kateryna FlyakBiology, Technion- Israel Institute of Technology

Cell-cell fusion plays an important role in the development of the nematode *C. elegans*. About one-third of the cells in *C. elegans* undergo fusion during some organs and tissue formation: pharynx, vulva, uterus, hypodermis and tail (Mohler et al., 2002).

Fusogens are the proteins that mediate this fusion process. There are two known fusogens in *C. elegans* that are both essential and sufficient for cell fusion: EFF-1 (Epithelial Fusion Failure 1) and AFF-1 (Anchor-cell Fusion Failure 1) (Mohler et al., 2002; Sapir et al., 2007).

C. elegans presents a unique model organism for studying cell-cell fusion events during hermaphrodite and male development. For instance, in the male tail region, the copulatory organ consists of a cuticular fan supported by nine pairs of rays. The formation of each ray begins at third larval stage (L3) with divisions of a ray progenitor cell (Rn; n=1-9) (Sulston et al., 1980). After two generations of divisions, the anterior daughter cell (Rn.a) will become the ray; the posterior daughter (Rn.p) of each ray progenitor cell tends to fuse either with other Rn.p cells (R1.p-R5.p) to create the male tail seam (SET), or with the major hypodermal syncytium hyp7 (R6.p-R9.p) (Sulston et al., 1980). As part of male tail morphogenesis, at the last larval stage (L4) the tail tip hypodermal cells, hyp8-hyp11, fuse together to form a cell with five nuclei (Nguyen et al., 1999). However, it is unclear for hyp8-hyp11 cells which fusogen(s) mediates their fusion (Mason et al., 2008), and unknown for Rn.p cells.

Characterization of *eff-1* mutant animals revealed a number of developmental abnormalities, including aberrant fan and ray formation in the male tail together with pointed leptoderan tail resembling the hermaphrodite tail (Mohler et al., 2002). A similar phenotype of smaller fan and shorter rays was observed in *uba-1* mutant animals (Kulkarni & Smith, 2008). UBA-1 is the only E1 ubiquitin-activating enzyme in *C. elegans*, which is the initiator in the ubiquitination cascade. A wide range of processes are regulated by ubiquitin, including the endocytosis-mediated downregulation of proteins (Woelk et al., 2007). Additionally, it has been shown previously that EFF-1 removal from the surface of hypodermal cells is mediated by dynamin and Rab-5-dependent endocytosis (Smurova & Podbilewicz, 2016).

Taking it all together, I raise the hypothesis that there is a ubiquitin-mediated regulation of cell-cell fusion during male tail morphogenesis.

418C **Uncovering molecular mechanisms for developmental synchrony with *in-vivo* spatial temperature perturbations in *C. elegans* larva** Eliot Schlang[1], Marie-Caroline Jullien[2], Simon Berger[3,4], Gregoire Clement[5], Wolfgang Keil[11]PCC-UMR168, Institut Curie, [2]UMR 6251, Université de Rennes, Institut de Physique de Rennes, [3]Department of Molecular Life Science, University Zürich, [4]Institute for Chemical and Bioengineering, ETH Zürich, [5]Gulliver UMR CNRS 7083, ESPCI Paris

The development of multicellular organisms is strikingly robust with respect to environmental fluctuations such as nutrient availability, oxygen level, or presence of pheromones. One example of this robustness is the fact that poikilothermic animals greatly change their rates of development as a function of ambient temperature without any loss in developmental precision and invariant developmental outcomes over wide temperature ranges. How this robustness is achieved is not well understood.

Here, we focus specifically on the adaption of developmental rate of cold-blooded animals to changes in temperature and ask to what extent cell-cell communication contributes to robust temperature scaling of development. Our approach is to subject developing *C. elegans* larvae to steep linear temperature gradients of about 10˚C/mm along their anteroposterior axis.

To this end, based on the microfluidic designs developed by Berger et. al. (2021) and the temperature control system relying on an optimized set of resistors micropatterned onto a coverslip developed by Selva et al. (2009), we engineered a novel microfluidic system that combines confinement of feeding and growing elongated larva with precise spatiotemporal temperature control at the scale of 10-50um. This enables long-term high-resolution in-vivo imaging of larvae developing in steep temperature gradients. Using this system, we uncovered that hypodermal stem cell division timings which normally occur synchronously through the anteroposterior axis in each larval stage, remain synchronized, even after prolonged exposure to strong temperature gradients. This striking result suggests that cell-cell communication underlies robust temperature scaling.

We are planning to investigate whether synchrony of developmental gene expression is also maintained in these conditions and other tissues, such as the somatic gonad. For instance, we have recently shown (Kinney, Sahu et. al. 2022) that *lin-4* microRNA transcriptional pulses in the hypodermis are highly synchronous. By imposing a temperature gradient, will the pulsed expression of *lin-4* and other microRNAs remain as synchronized as the seam cell division timings?

Our results provide a quantitative and well-controlled approach to understanding the molecular mechanisms underlying the coordination and synchronization of *C. elegans* post-embryonic development.

419C **Non-canonical DAF-2/IR signalling regulates transient larval arrests in C. elegans** Francisco J Romero-Expósito[1], Francesca Sartor[2], Nicola Gritti[3], Martha Merrow[2], Jeroen S van Zon[3], Alejandro Mata-Cabana[1], María Olmedo-López[11]Department of Genetics, University of Sevilla, Spain, [2]Institute of Medical Psychology, Faculty of Medicine, LMU Munich, Germany, [3]AMOLF Institute, The Netherlands

Insulin/IGF1-like signalling (IIS) is one of the key pathways that control *C. elegans* postembryonic development. Signalling through the DAF-2/Insulin receptor (IR) links development to food availability, slowing or even arresting development when there is a lack of nutrients. Most *daf-2* phenotypes are mediated by nuclear relocalization of DAF-16/FoxO, the major effector of the IIS pathway. By performing continuous monitoring of *C. elegans* development, we have revealed a transient larval arrest at the beginning of larval stages that is controlled by the DAF-2 receptor. This previously uncharacterised process occurs at the beginning of larval stages L2, L3, and L4, being most relevant at the beginning of the second larval stage (transient L2 arrest, L2a). Transient larval arrest also occurs in wild-type animals, but its frequency and duration increase in *daf-2(e1370)*. Interestingly, this phenotype is partially independent on DAF-16/FoxO. We have analysed the effect of mutations in varied components of insulin signalling to find at what level the signalling from DAF-2 bifurcates from the canonical pathway, finding relevant roles for DAF-18/PTEN and MPK-1/ERK1,2 in the process.

In order to understand what provokes the transient larval arrest we have focused on seam cells, hypodermal cells that divide symmetrically at the beginning of L2, in addition to showing an asymmetrical division at the beginning of each larval stage. In wild-type worms, the L2 symmetric division of these cells occurs during the moulting phase of the worms. In the case of *daf-2(e1370)* mutants, the timing of symmetric division is not well defined, sometimes occurring after the ecdysis, with delays specifically in the divisions of the V1 and V6 cells. We observed that worms whose V1 and V6 had not divided before the end of the moulting phase entered into L2 transient arrests. Altogether, our results suggest that low DAF-2/IR signalling reduces the synchrony between moulting and seam cell divisions, potentially leading to transient larval arrest.

420C **Genetic dissection of *in vivo* direct cellular reprogramming** Ismail Özcan, Anna Reid, Margaux Quiniou, Amin R. Shadfar, Baris TursunMolecular Cell Biology Unit, Institute of Cell and Systems Biology of Animals, University of Hamburg

Dissecting cell fate regulatory mechanisms in the context of cellular reprogramming is central to developing strategies that ensure the quality and safety of reprogrammed cells for medical applications. The importance of different regulatory pathways and how the original cell fate is shut down while establishing the new cell fate during reprogramming are not fully understood. To address these questions, we developed a novel system where coelomocytes (CCs), which have scavenging and hepatic function in *C. elegans,* can be reprogrammed to intestinal or neuronal-like cells upon overexpression of the GATA-type transcription factor (TF) ELT-7 or the ZNF-type TF CHE-1, respectively. Reprogramming of CCs is accompanied by loss of CC gene expression and physiological functions as is expected for faithful cell identity conversion. We performed an RNAi screen targeting chromatin regulators/remodelers to identify CC reprogramming enhancers/suppressors. We obtained numerous conserved reprogramming factors, which when knocked down either enhance or suppress CC reprogramming to neuron-like cells. Our results show that depletion of H3/H4 core histone genes and components of the conserved SET1/MLL complex significantly enhance CC reprogramming. On the other hand, depletion of the effector protein Argonauts, which are highly conserved key regulators in all small-RNA-guided silencing processes, suppresses CC reprogramming. In addition, the members of the 22G RNA pathway including *sago-1* and *sago-2* and RDE-10/RDE-11 complex, as well as 26G RNA pathway components such as *ergo-1*, were also identified to be required for CC reprogramming as their knockdown suppresses CC reprogramming. These findings reveal the implication of effector protein Argonauts in TF-mediated direct reprogramming of CCs and suggest that small regulatory RNAs may be involved in CC cell fate maintenance. Furthermore, we applied CC-specific RNA-seq, ATAC-seq, and ChIP-seq to uncover transcriptomic and chromatin accessibility changes during CC reprogramming. Profiling the changes in chromatin accessibility in wild-type and reprogrammed CCs at three different time points indicates that natural CCs possess more nucleosome-free chromatin regions compared to reprogrammed CCs. Our results suggest that abundantly accessible chromatin regions make CCs amenable to TF-mediated direct reprogramming.

421C **Understanding the mRNA regulatory repertoire of LIN-41 during cell fate decisions** Benedicte Storvik Nordhagen[1], Pooja Kumari[2], Rafal Ciosk[21]Biosciences, University of Oslo, [2]Bioscience, University of Oslo

LIN41 proteins are prominent regulators of cellular plasticity, which control either the stability or translation of specific mRNAs. The *C. elegans* LIN-41, for example, induces mRNA decay when bound to the 3'UTR but translational repression when bound to the 5'UTR of somatic mRNAs. In both cases, the RNA binding is mediated by the NHL domain that, in LIN41 proteins, recognizes a stem-loop RNA element, whose shape determines the RNA-binding specificity. In addition to regulating proliferation versus differentiation decisions in somatic epidermal stem cells, LIN-41 functions in the germline, where it controls several aspects of the oocyte-to-embryo transition, including reprogramming into pluripotency. Surprisingly, in contrast to the RNA-binding mechanism described in the soma, the specificity of LIN-41 towards some germline mRNA targets is mediated via another RNA-binding protein associated with LIN-41, rather than the RNA stem-loops. We are dissecting the underlying RNA regulatory mechanisms in the soma and germline. Towards that we have identified the protein partners of LIN-41 and are performing screens to determine their roles in regulation of different mRNA targets in different tissues. Our results will help to explain on LIN-41 expands its regulatory repertoire and deploys distinct RNA-repressing mechanisms.

422C **Parental dietary restriction delays offspring growth due to suboptimal ribosome provisioning** Sigma Pradhan,

Klement Stojanovski, Benjamin TowbinInstitute of Cell Biology-University of Bern

Environmental conditions can impact the phenotype of subsequent generations, but the molecular mechanism underlying such intergenerational plasticity is poorly understood. Here, we studied the effects of parental dietary restriction (DR) on offspring growth. Using time lapse microscopy of individual animals, we found that animals hatching under well-fed conditions have a growth delay when their parents experienced DR. This growth delay coincided with a reduced ribosome concentration during the first hours after hatching. During L1 development, offspring of DR parents upregulated ribosome synthesis. By the L2 stage, they thereby reached the same ribosome level and growth rate as progeny of well-fed parents. Partial experimental degradation of ribosomes in the germline of well-fed parents similarly resulted in a growth delay of their progeny, suggesting a causal relationship between ribosome provisioning and the rate of progeny development. Indeed, the concentration of ribosomes at hatch was predictive for the heterogeneity in L1 duration observed among isogenic individuals. Together, we characterize maternal ribosome provisioning as a parsimonious mechanism for diet-induced intergenerational plasticity.

423C **Transcriptional control of male tail tip morphogenesis by DMD-3** Porfirio Fernandez, Karin Kiontke, Christopher Isaacs, Yash Patel, David H.A. FitchBiology, New York University

How transcriptional regulators are linked to cell biological effectors of morphogenesis is not fully understood. To elucidate this linkage, we use the *C. elegans* male tail-tip as a model. The tail-tip is made of 4 cells which in males undergo changes in shape and position during the L4 stage. The transcription factor DMD-3 is hypothesized to be the main regulator of this Tail-Tip Morphogenesis (TTM). To identify genes involved in TTM, the lab has performed a whole-genome RNAi screen and an RNA-seq analysis of tail tips isolated from L4 wild-type and *dmd-3* mutant males. Differential expression (DE) analysis of these data yielded candidates for genes regulated by DMD-3 directly and indirectly. To find direct targets of DMD-3, we conducted male-specific DMD-3 ChIP-seq on whole worms during TTM. We found 1755 DMD-3 peaks representing 3636 genes and a putative DMD-3 binding site. Integrating these data with the DE analysis, we found 147 candidates for direct DMD-3 targets in tail-tip cells. To validate genes, we inserted GFP into the endogenous loci using CRISPR and evaluated the expression of the fusion proteins. We then deleted the sequences associated with DMD-3 ChIP peaks using CRISPR and assessed if this resulted in an altered expression or a TTM phenotype. So far, we found 4 genes where deletion of a peak had an effect: (1) The transcription factor FOS-1 is expressed in nuclei of male tail tips and the vulva. Deletion of the DMD-3 peak site 5› to the *fos-1* gene specifically abolishes expression in male tail tip nuclei. (2) NMY-2 is localized in a “cap” at the end of the retracting tail tip. Removal of its DMD-3 peak dramatically reduces tail tip expression. (3) PAN-1 is first seen in puncta at the cell surface, then at the nuclear envelope, and later in the nucleus. The *pan-1* locus has 2 DMD-3 peaks. Removal of one has little effect on protein expression, but 10% of males show TTM defects. Removal of both peaks leads to larval arrest. (4) The E-cadherin HMR-1 is expressed at adherens junctions at the onset of TTM and later forms puncta at the cell surface. Deleting its DMD-3 peak has little effect on expression but results in epidermal bulges in both sexes. This indicates that the peak region contains binding sites for additional factors. We are currently working on narrowing down the DMD-3 binding regions and identifying and validating a specific binding motif. This will further help identify the genes directly regulated by DMD-3.

424C **Identifying co-factors that drive TRA-1 activator function** Jibran Imtiaz, Youngquan Shen, Ronald EllisMolecular Biology, Rowan University SOM

Gli proteins 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 cancer. However, the mechanisms by which they interact with co-factors are poorly understood. We are analyzing co-factors of Gli proteins using TRA-1 in *Caenorhabditis* nematodes. The TRA-1 zinc fingers are structurally like those of other Gli proteins, and TRA-1 can be cleaved like other Gli proteins to form a repressor. However, its function has changed during evolution — in nematodes, TRA-1 controls sexual fates and plays a central role in self-fertility, which makes it easy to assay mutant phenotypes. Furthermore, worms lack classical Hedgehog signaling, so study of nematode TRA-1 should reveal other types of regulation.

Our lab has shown that full-length TRA-1 can work as an activator and promote spermatogenesis, and that the mutation *cbr-tra-1(v48)* disrupts this process and prevents spermatogenesis. We suspect that regulation of TRA-1 activator plays a major role in the evolution of hermaphrodite spermatogenesis in nematodes. Because *v48* was isolated in a classical EMS mutagenesis, we recently made other activator mutations to confirm that all of its phenotypes were due solely to the alteration of TRA-1.

Since TRA-1 activator is likely to interact with a diverse set of co-factors, whose activities might help determine whether specific targets are activated or repressed, we identified sites in TRA-1 where an OLLAS tag does not inactivate the protein, and used anti-OLLAS antibodies to isolate TRA-1 complexes. We are now characterizing the products, and preparing to analyze them with mass spectrometry. While doing so, we will look for important modifications to TRA-1 itself, as well as the precise site of cleavage

that forms the repressor. In addition, we hope to identify TRA-1 co-factors and learn how they regulate Gli activity. Finally, we will see if any of these co-factors has a novel role in species that produce self-fertile hermaphrodites.

425C **DAF-16/FOXO inhibits NHR-23/ROR and the *let-7* family of microRNAs during dauer in *C. elegans*** Himani Anand Galagali[1], Matthew J Wirick[2], Amelia F Alessi[1], Margaret R Starostik[1], Mindy Clark[1], Laurianne Pene[2], Priya Balamurugan[1], Shreya Singireddy[1], Suhua Feng[3], Ruhi Patel[3], Steven E Jacobsen[3], Alison R Frand[3], Xantha Karp[2], John K Kim[11]Department of Biology, Johns Hopkins University, [2]Department of Biology, Central Michigan University, [3]Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles

Animals have evolved adaptive mechanisms to cope with varying environmental conditions. In *C. elegans*, starvation and overcrowding induces L2 larvae to enter the quiescent, stress-resistant dauer stage. Here, we report the interactions between the conserved transcription factors DAF-16 and NHR-23 (homologs of mammalian FOXO and RORs, respectively) and the *let-7* family of microRNAs as key determinants in the transition from continuous development to the stress-induced dauer stage. The *let-7* microRNA was first identified as a master regulator of the L4 to adult transition (Reinhart *et al*, 2000) and later as a deeply conserved promoter of differentiation (Paquinelli et al, 2000). In *daf-7(e1372)* mutants that form constitutive dauers, introducing a loss-of-function *daf-16* mutation results in transcriptionally elevated *let-7* levels that, in turn, lead to the precocious expression of an adult-specific collagen marker, COL-19::GFP, in seam and hypodermal cells. Overexpression of *let-7* under a dauer-specific promoter is sufficient to induce COL-19::GFP expression in *daf-7(-)* dauers alone. Our ChIP-seq analysis reveals that DAF-16 binds upstream of the *let-7* transcriptional activator, *nhr-23*. Furthermore, *daf-16(-); daf-7(-)* dauers have elevated levels of *nhr-23* mRNA and NHR-23 protein. These data suggest that upon dauer entry, DAF-16 binds upstream of *nhr-23* and inhibits its transcription, thereby downregulating *let-7* transcription and maintaining multipotency. As NHR-23 is a master transcription factor regulating a host of genes required for animal molting, we hypothesize that attenuating *nhr-23* expression by DAF-16 may facilitate pausing of the molting cycle during dauer. Comparative analysis of DAF-16 ChIP-seq and mRNA-seq in *daf-16(-); daf-7(-)* vs *daf-7(-)* (Wirick *et al*, 2021) identifies 287 additional genes that may be directly inhibited by DAF-16 in dauers. These putative DAF-16 targets are enriched for pro-growth genes required for mitotic DNA replication and cuticle development. Taken together, our study supports a model where DAF-16 plays a dual role in not only activating stress response pathways, but also concomitantly repressing *nhr-23*- and *let-7*-mediated gene programs for differentiation and continuous development during dauer.

426C **The C. elegans intestine as a model to study the function of polyploidy** Christa Jordan Ortiz, Noam Perry Levy, Nienke Vollebregt, Matilde GalliHubrecht Institute

Most eukaryotic cells contain two pairs of homologous chromosomes, however, polyploid cells, which contain more than two sets of chromosomes, are widely present within many organs of animal and plant species. Polyploid cells are often found in tissues that have critical functions to generate nutrients and metabolites for their surroundings, such as the mammalian liver and placenta. Although polyploidy has long been associated with increased cellular growth and metabolic output of cells, surprisingly little is known on how having additional copies of the genome actually influences cellular and tissue homeostasis. Specifically, it is largely unknown how polyploidy affects RNA transcription, protein synthesis and cell growth rates. The *C. elegans* intestine provides a powerful system to study the function of polyploidy, as polyploid cells arise at defined moments in development, and can be studied using quantitative microscopy and genetic perturbations. By performing smFISH on intestinal cells of different ploidies, we are characterizing how polyploidization influences mRNA and rRNA transcription. Additionally, we have developed an AID (Auxin Induced Degradation)-based system to inhibit polyploidization of intestinal cells in a time- and tissue-specific manner. We find that animals with reduced intestinal ploidy have largely reduced brood sizes, likely due to reduced vitellogenin expression. Interestingly, we also find that inhibition of intestinal polyploidy results in reduced male fertility due to decreased sperm production and activation. Together, this work will help us unravel the cellular and organism-wide consequences of polyploidization.

427C **Modified Mechanisms of Chromosome Inheritance in the Trioecious Nematode *Auanema rhodensis*** Liesl G Strand[1], Pablo Gonzalez de la Rosa[2], Sally Adams[3], Mark L Blaxter[2], Andre Pires da Silva[3], Anne M Villeneuve[41]Developmental Biology, Stanford University, [2]Tree of Life, Wellcome Sanger Institute, [3]School of Life Sciences, University of Warwick, [4]Departments of Developmental Biology and Genetics, Stanford University

Auanema rhodensis is an unusual three-sexed nematode species in which XX females, XX hermaphrodites, and XO males coexist in natural populations. *A. rhodensis* also exhibits unusual patterns of sex chromosome inheritance, driven in part by alterations of the oogenic meiotic program that result in the production of haplo-X oocytes in females, but nullo-X oocytes in hermaphrodites. We set out to explore the mechanisms underlying this meiotic variation by examining the cytological distribution of histone modifications associated with active and repressive chromatin, which revealed that *A. rhodensis* displays distinct patterns of germline and chromosome organization during meiotic prophase that diverge from those found in the well-studied Caenorhabditis genus. Notably, sex chromosome organization in both hermaphrodites and females appears distinct from the

autosomes, with the X chromosomes being highly enriched for the repressive chromatin mark H3K9me3. Despite enrichment for this repressive mark, however, the X chromosomes appear much larger than the autosomes during diakinesis, when chromosomes condense in preparation for the meiotic divisions. This finding was unexpected based on the previously-published *A. rhodensis* short-read genome assembly, which had indicated an X chromosome half the size of autosomes. The basis of this discrepancy became clear following a new genome assembly derived from accurate long-read sequencing and Hi-C data, which revealed that extensive blocks of repetitive DNA are eliminated from the somatic genome during development. Using FISH probes targeted to germline-specific and somatic-retained repeat sequences, we confirmed the unusual patterns of meiotic sex chromosome inheritance inferred from the genetic data. Moreover, we have established that programmed DNA elimination (PDE) in somatic cells occurs subsequent to the 8-cell stage, and that the bulk of PDE is already completed by the 32-cell stage. We are intrigued by the possibility that the large repetitive chromosomal regions maintained in the germ line of this organism may play structural or functional roles that contribute to the atypical segregation patterns observed during spermatocyte and oocyte meiosis.

428C **The asymmetric cell division of a glial neuronal progenitor in *Caenorhabditis elegans* is co-regulated by PIG-1/MELK and a Wnt/☐-catenin asymmetry pathway** Joseph Gehler, Carla Lloret Fernandez, Victoria Rowe, Michele Sammut, Richard PooleCell & Developmental Biology, UCL

Generating asymmetric cell divisions is a universal requirement for all multicellular life. As a result, the regulatory paradigms which facilitate division asymmetry are thought to be highly conserved. Such paradigms are understood to operate on multiple levels, including by mediating transcriptional as well as non-transcriptional asymmetry between daughter cells. In this study, I investigate the molecular regulators of transcriptional and nontranscriptional asymmetry in the recently discovered neurogenic asymmetric cell division of a glial neuronal progenitor in *C. elegans*. In this division, a glial mother cell (the amphid socket or AMso) re-enters the cell cycle during sexual development of the male in order to generate an anterior glial daughter and a posterior neuronal daughter (the Mystery Cell of the Male or MCM interneuron).

In this study, I demonstrate that the mutant allele *drp9*, generated by an unbiased forward genetic screen, renders the AMso division symmetric (the posterior daughter fails to adopt MCM identity). Whole-genome sequencing and complementation testing reveals *drp9* is a novel PIG-1/MELK loss-of-function allele. Additionally, I use an RNAi screen to demonstrate that a Wnt/☐-catenin asymmetry pathway involving a number of highly conserved elements co-regulates the asymmetric division of the amphid socket and subsequent adoption of MCM terminal identity by the posterior daughter cell. Notably, perturbations to these elements render the AMso division symmetric by impairing MCM identity acquisition in the same manner as PIG-1 abrogation. Therefore, the acquisition of terminal neuronal identity by the MCM, a neuron produced via the asymmetric cell division of a glial neuronal progenitor, relies on both PIG-1 as well as activation by Wnt transcriptional effector POP-1/TCF, which is, in turn, regulated by and reliant upon a number of upstream factors which have previously been implicated in canonical Wnt asymmetry signaling, including ☐-catenins, Frizzled receptor orthologues, and ROR ortholog CAM-1. We are currently examining the possibility of direct interactions between PIG-1 and the Wnt asymmetry pathway.

429C **Regulation of programmed cell death during *C. elegans* development** Chloe Emerson, Alison Kochersberger, Marc Hammarlund Genetics, Yale University

Programmed cell death (PCD) is a highly conserved process that removes healthy cells during development. The apoptotic mechanisms that lead to cell death during PCD are well described, but the signaling processes that initiate this pathway are incompletely characterized. In *C. elegans*, PCD is initiated by transcription of *egl-1*, and carried out by a cascade of caspases including CED-3. The regulation of *egl-1* that determines if a cell will live or die is not fully understood. We developed a novel fluorescent reporter that indelibly marks cells that express *egl-1*, allowing PCD events to be studied in *ced-3* mutant animals, in which PCD is inhibited. Our reporter is based on the Q system in which the transcription factor QF activates reporters downstream of the QUAS enhancer, and which has previously been adapted for use in *C. elegans* (Wei et al., 2012). We used CRISPR to insert an SL2-QF sequence into the *egl-1* locus, which causes QF to be co-transcribed with *egl-1* in cells initiating PCD. In a separate transgene, we used a similar SL2 to transcriptionally couple GFP and QF, this time under the control of QUAS. Combining these two components creates a positive feedback loop which results in continuous expression of GFP in cells that transcribe *egl-1*; we call this reporter "*egl-1:Q-loop.*" Despite *egl-1* transcription occurring transiently in the embryo, fluorescent signal in *egl-1:Q-loop ced-3* worms persists throughout the lifetime of the worm. To identify novel factors that regulate *egl-1* expression, we performed a forward genetic screen in the *ced-3* mutant background. We found several mutants that result in altered *egl-1:Q-loop* expression. We expect that identifying and characterizing these genes will result in new information about mechanisms that control *egl-1* and PCD. Using modified versions of our reporter, we also plan to explore the fate and function of 'undead' cells in *ced-3* mutant worms, including the impact these cells have on neuronal wiring and behavior. By discovering the molecular mechanisms regulating PCD, we will identify fundamental biological pathways and regulatory mechanisms that underlie a critical

developmental cell fate decision.

430C **Transcriptional Regulation of *hlh-2* in the Larval Somatic Gonad** Jee Hun Kim, Iva Greenwald Biological Sciences, Columbia University in the City of New York

The transcription factor HLH-2 plays multiple roles in the specification and function of the anchor cell (AC) of the hermaphrodite somatic gonad. Z1 and Z4 each give rise to two α cells (Z1.ppp and Z4.aaa) and two β cells (Z1.ppa and Z4.aap). These four cells are initially competent to become the AC; the β cells soon commit to the Ventral Uterine precursor cell (VU) fate while their sister α cells undergo the LIN-12/Notch-mediated AC/VU decision to resolve which will be the AC and which will be a VU.

HLH-2 is first observed in Z1.pp and Z4.aa, and initially in their daughters, the α and β cells. Transcriptional and post-translational regulatory mechanisms restrict HLH-2 to the presumptive AC. We reported evidence for LIN-12/Notch-mediated degradation of HLH-2 and for LIN-12-independent negative regulation of *hlh-2* transcription in the β cells (Benavidez, Kim & Greenwald 2022). Here, we are exploring two aspects of *hlh-2* transcriptional control: (1) which elements of the *hlh-2* 5' regulatory region regulate transcription and (2) how the Wnt pathway regulates *hlh-2* transcription.

The 5' regulatory region of *hlh-2* contains *hlh-2*prox, a 327 bp element necessary and sufficient for *hlh-2* expression specifically in α and β cells and their parents in the hermaphrodite gonad (Sallee & Greenwald 2015; Attner et al. 2019). Therefore, this element is crucial for integrating spatial, sexual, and temporal cues for *hlh-2* transcription in α and β cells. To identify functional elements within *hlh-2*prox, I generated single-copy insertion transgenes with deletion mutations in the LGI docking site using CRISPR/Cas9. As HLH-2 is also part of a "bHLH code" for gonadal regulatory cell fates (Sallee et al. 2017), I am also investigating other areas of the *hlh-2* 5' regulatory region that may mediate inputs into *hlh-2* expression in the other regulatory cells.

The Wnt/β-catenin asymmetry (WβA) pathway may be involved in LIN-12-independent difference in *hlh-2* transcription between the α and β cells (Sallee et al. 2015), and endogenous levels of GFP::POP-1/TCF expression are higher in the α cells than β cells. Although uniform overexpression of GFP::POP-1 (using Flexon: Shaffer and Greenwald 2022) did not alter the restriction of HLH-2 to the AC or the AC/VU decision, *lit-1(RNAi)* causes supernumerary HLH-2-expressing cells that resemble ACs. I am performing tissue-specific RNAi (based on Flexon) and AID to elucidate the role of the WβA pathway in *hlh-2* expression and AC/VU decision.

431C **Genetic analyses of PGL proteins using newly generated CRISPR alleles** Devavrat Bodas, Geraldine Seydoux Johns Hopkins School of Medicine / HHMI

P granules are perinuclear condensates in *C. elegans* germ cells proposed to serve as hubs for surveillance of germline transcripts by Argonautes. PGL-1,2 and 3 are P granule proteins whose loss leads to temperature-sensitive sterility (Kawasaki et al., 2004). PGL proteins share a common structure: two N-terminal dimerization domains followed by a disordered region. PGL-1 and PGL-3 are most similar and contain a C-terminal RGG repeat domain predicted to bind RNA. PGL-2 is most divergent and lacks the RGG domain. PGL proteins form homo and heterodimers and PGL-3 is sufficient to form condensates *in vitro* (Kawasaki et al., 2004, Putnam et al., 2019).

To examine the function of PGL proteins, we generated tagged and predicted null alleles at each locus. We found that all three PGLs co-localize in P granules throughout germline development and depend on each other for maximum enrichment in P granules. Zygotic expression of each PGL protein contributes to fertility at high temperature with the triple mutant nearly 100% sterile at 25°C. Curiously, PGL-2 appears to be toxic when expressed in the absence of PGL-1 and PGL-3. The *pgl-1; pgl-3* double mutant is 100% sterile at 20°C in contrast to the triple mutant, which is sub-fertile and can be propagated for at least 3 generations at 20°C. We find that polyA RNA still enriches in perinuclear foci in the triple mutant, suggesting that PGL proteins are not essential to retain mRNAs in P granules. Our results favor a model whereby condensation of PGL proteins ensure proper functioning of Argonautes and other small RNA pathways especially at high temperature. We will present our ongoing investigations as to the origin of the sterility of *pgl* mutants.

432C **Building a developmental proteomics map of *Caenorhabditis elegans* tissues** Emmanuel K Fiagbedzi, Dhanya Cheerambathur, Georg Kustatscher University of Edinburgh

Proteomic alterations are a key element in the formation of specialized cells and tissues during the development of a living organism. Many diseases are attributed to perturbations in the protein composition during development. Tissue specific transcript expression is only a moderate predictor for protein abundance, as it does not account for post-transcriptional processes such as translational regulation or protein stability. A tissue-specific temporal proteomics map of *C. elegans* development has not been described yet. Previous proteomic approaches have either been limited to the whole organism or a specific tissue at certain developmental stage. Here we aim to develop a proteomics approach to track protein abundance changes in a tissue-specific manner during development. This approach combines fluorescence-activated cell sorting (FACS) to isolate and enrich

for specific tissues, and analysis using a highly sensitive proteomics strategy called data independent acquisition on an Orbitrap Exploris mass spectrometer. Using this approach we can detect around 5500 proteins from 25000 FACS-sorted cells. We began by focusing on the neuronal tissues during embryonic development and are applying the approach to analyse the proteome of the embryonic neurons. Overall, we expect the tissue-specific data revealed using this approach to further functional studies of yet uncharacterized proteins and provide more insight into how developmental disorders occur.

433C **LIN-46 post-translationally down-regulates HBL-1 to specify seam cell fate by potentially influencing chromatin states during larval development** Reyyan Bulut, Victor AmbrosUniversity of Massachusetts Chan Medical School

Developmental cell fates are enforced by cell type specific gene expression programs. Lineage specific transcription factors establish cell type specific gene expression patterns by binding to their specific cis-regulatory elements in the chromatin. During *Caenorhabditis elegans* larval development, hypodermal stem cells (seam cells) execute an asymmetric (self-renewal) division before each molt. In addition, certain seam cell lineages divide symmetrically at the end of L1 molt, doubling their number. This developmental cell division fate is specified by the lineage specific transcription factor HBL-1. Mutations that prevent the timely down-regulation of HBL-1 by the end of L2 result in abnormal seam cell numbers. Previous research described that HBL-1 activity is inhibited by LIN-46 through a post-translational mechanism during L2d and dauer interrupted developmental trajectories. Sequence homology groups LIN-46 with evolutionarily conserved molybdenum cofactor (Moco) biosynthesis enzymes. In *C. elegans*, Moco biosynthesis is carried out by the LIN-46 paralog MOC-1. Our evolutionary analysis of 58 *Caenorhabditis* genomes indicates that *lin-46* evolved in the ancestral *moc-1* locus after a duplication event. To investigate the putative functional divergence of LIN-46 and MOC-1, we performed rescue experiments in *moc-1* and *lin-46* mutant worms. Ectopically expressing *moc-1* using *lin-46* regulatory sequences did not rescue *lin-46* loss-of-function phenotype. Furthermore, ectopic expression of *lin-46* with *moc-1* regulatory sequences not only did not rescue *lin-46* loss-of-function phenotypes but also did not phenocopy *lin-46* gain-of-function. We performed yeast-two-hybrid (Y2H) screens to identify putative physical interactors of MOC-1, LIN-46 and HBL-1. While MOC-1 did not have common interactors with LIN-46 or HBL-1, LIN-46 and HBL-1 screens isolated common interactors. We tested the involvement of these common interactors in seam cell fate specification by knocking down the corresponding genes in a sensitized genetic background that develops through the L2d. This strategy identified two previously known chromatin remodeling factors and a novel gene as seam cell fate regulators. Lastly, our one-by-one Y2H strategy showed that LIN-46 physically interacts with HBL-1 in yeast. Taken together, our findings suggest a role for LIN-46 as a post-translational HBL-1 regulator that possibly influences chromatin states underlying cell fate transitions.

434C **The conserved Toll-like receptor homolog *tol-1* is involved in neuroblast migration during *C. elegans* ventral epidermal morphogenesis** Zoe Tesone[1], Jeff Hardin[2], Nathalie Pujol[31]Cellular and Molecular Biology Graduate Program, University of Wisconsin-Madison, [2]Department of Integrative Biology, University of Wisconsin-Madison, [3]CIML, Aix Marseille Univ

Two crucial events at the end of gastrulation in the *C. elegans* embryo are the closure of the ventral cleft and ventral epidermal enclosure. Cleft closure involves the formation of two rosettes with apical enrichment of HMP-1/α-catenin, as ventral neuroblasts migrate towards the midline and seal the surface opening left behind by internalizing cells[1, 3]. An hour later, ventral epidermal enclosure proceeds as epidermal cells migrate over ventral neuroblasts, led by four "leading cells," followed by sealing of a posterior ventral pocket via a purse string closure[2]. Actin and myosin localize to foci in both epidermal cells and neuroblasts to aid in the facilitation of ventral enclosure[4]. Given the similarities of cleft closure and ventral enclosure to movements in other animal embryos, we sought to identify novel, conserved molecular components involved in these processes. The only previously characterized role for the single *Toll* homolog (*tol-1*) during *C. elegans* development is in an avoidance behavior to pathogenic bacteria[5] and the development of the CO_2-sensing BAG neuron[6]. However, at 15°C, *tol-1 (nr2013)* deletion homozygotes exhibit less than 10% survival, with lethality at different developmental stages due to morphogenetic defects. We show that many *tol-1 (nr2013)* homozygotes are defective in neuroblast migration and rosette formation during cleft closure and/or ventral enclosure. *tol-1 (nr2013)* homozygotes display abnormal clustering of HMP-1/α-catenin to the vertices of neuroblast cells in rosettes. Endogenously tagged TOL-1 is expressed in ventral neuroblasts during cleft closure and ventral enclosure. We are investigating which cell types TOL-1 functions in through tissue-specific rescue experiments in epidermal cells and neuroblasts. We are also currently examining actomyosin mediated events during cleft closure and ventral epidermal enclosure in *tol-1 (nr2013)* mutants. We propose TOL-1 functions to promote rearrangement of neuroblasts into a rosette-like pattern, which in turn affects epidermal morphogenesis. Our work identifies a new role for TOL-1 in tissue morphogenesis and neuroblast migration during a fundamental developmental process in metazoans.

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435C ***sea-2* and *lin-66* are accessory heterochronic genes that come into prominence during L2d interrupted developmental trajectory** Reyyan Bulut, Victor AmbrosUniversity of Massachusetts Chan Medical School

During L1 molt in *Caenorhabditis elegans* larval development, a subset of hypodermal stem cells (seam cells) execute a symmetric division fate, increasing the total number of seam cells from 10 to 16. The most proximal regulator of this developmental event is the transcription factor HBL-1. Mutations that cause ectopic expression of HBL-1 after L2 stage result in animals with extra seam cells, whereas mutants that precociously down-regulate HBL-1 before the end of L2 stage end up with too few. Previous studies have identified two non-redundant and parallel mechanisms for HBL-1 down-regulation: 1) post-transcriptional silencing via microRNA complementary sites in the *hbl-1* 3'UTR, and 2) post-translational regulation of the nucleo-cytoplasmic partitioning of HBL-1 mediated by LIN-46. Moreover, the RNA binding protein LIN-28 regulates these pathways by repressing both *lin-46* and the microRNAs that target *hbl-1* transcript. Importantly, animals primarily engage the first mechanism when developing continuously under replete growth conditions, whilst the second mechanism predominates during L2d interrupted development. Focusing on the second mechanism, we tested previously identified negative regulators of *lin-28* for their involvement in HBL-1 regulation by knocking down their expression in animals that undergo L2d interrupted development. We found that knock-down of the *lin-28*-negative regulators *sea-2* and *lin-66* resulted in significant increase in the seam cell numbers during L2d interrupted development. Further epistasis analyses suggested that *sea-2* potentially functions by opposing a novel *lin-28/hbl-1* regulatory axis, whereas *lin-66* may mediate *hbl-1* down-regulation downstream of *lin-28* and *lin-46*. We tested the previously proposed dependency of *lin-66* on *lin-28* 3' UTR microRNA complementary sites for HBL-1 regulation using endogenous *lin-28* 3'UTR deletion alleles. Our findings suggested that while lin-66 does not function through the microRNA complementary sites, *lin-66* loss-of-function synergizes with *lin-28* gain-of-function alleles to promote ectopic HBL-1 expression and prevent the execution of adult specific fates in the hypodermis. We are currently investigating the impact of the knock down of *lin-66* and *sea-2* on HBL-1 nucleo-cytoplasmic partitioning.

436C **Targeting *C. elegans* Cyclic Nucleotide Phosphodiesterases as Potential Nematicides Using Chemical and Molecular Agents** Kranti K Galande, Kevin D Schuster, John J Collins, Rick H CoteMolecular Cellular & Biomedical Sciences, University of New Hampshire

Plant parasitic nematodes are responsible for ~$100B in annual crop losses. Current chemical controls are only partially effective and pose significant adverse consequences for human health and the environment. There is evidence that cyclic nucleotide signaling pathways are critical for regulating nematode behavior and developmental progression, but the phosphodiesterases (PDEs) and cyclases responsible for regulating many of these physiological processes are poorly characterized. We have undertaken three complementary strategies to evaluate the role of the six nematode PDE genes [orthologous to vertebrate PDE1-4 (nematode *pde-1 to pde-4)*, PDE8 (*pde-6)* and PDE10 (*pde-5)*] that are present throughout the nematode phylum [Shuster et al., (2019) PLoS ONE 14(3): e0214554] using the free-living nematode *C. elegans* as a model system: (1) generation of transgenic strains containing an ablated PDE gene; (2) RNAi knockdown; and (3) exposure to PDE inhibitors designed to target human orthologs of *C. elegans pde-1* through *pde-6*. We find that CRISPR/Cas9 ablation of each of the six PDE genes resulted in behavior and developmental progression indistinguishable from the N2 wildtype strain with the following exceptions: (1) deletion of *pde-1* disrupted the chemotactic response to the attractant butanone (as did exposure of wildtype nematodes to the human PDE1-specific inhibitor PF-04471141); (2) whereas a *pde-1/pde-4* double knock-out was phenotypically indistinguishable from the N2 strain, knocking down pde-3 expression by RNAi in the double knock-out resulted in lethargy, developmental delays, and greatly reduced fecundity. In addition, exposure of *C. elegans* to the human PDE10 inhibitor MP-10 retarded growth and reduced viability and fecundity (although the *pde-5* knockout strain was not similarly affected). Consistent with previous work, we hypothesize that many (but not all) cyclic nucleotide cell signaling pathways in *C. elegans* are regulated by multiple PDEs. We conclude that a combination of chemical and genetic controls targeting nematode PDEs has the greatest potential to disrupt the phytoparasitic nematode lifecycle and minimize adverse effects on non-target animals and plants, thereby enhancing sustainable agricultural practices. Supported by USDA/NIFA through the NH Agricultural Experiment Station grant NH00679.

437C **Cell focusing and the tumor necrosis factor superfamily** Christian Wartenberg[1], Christian Hennig[2], Ralf Schnabel[21]Developmental Genetics, Institut fuer Genetik, Technische Universitaet Braunschweig, [2]Institut fuer Genetik, Technische Universitaet Braunschweig

Pattern formation in the embryo is a fundamental basis for the creation of biological form. Despite the finally stereotypic development, extensive cell migrations are sorting out the variable cell positions caused by the cell divisions (Schnabel et. al., 1994). The positions acquired by cells at the premorphogenetic stage depend on their specific cell fates. Extensive cell fate/identity changes caused for example in *glp-1* or *apx-1* mutant embryos result in dramatically altered cell positions. However, these correspond to the WT position of the cells with the same identity, which are reached by extensive cell migrations. (Schnabel et. al., 2006). We now identified two unique genes playing essential roles in this cell focussing process. These feature highly conserved cysteine rich regions typical for the tumor necrosis factor superfamily domain (Conserved domain database, NCBI). Both genes are expressed in the embryo (scRNAseq data, Packer et. al., 2019). M01G5.1 is expressed ubiquitously. All cells of the mutant embryo are unable to migrate in a directed manner, they miss the normal position (91.9% general, 61.0% effective migration of WT), suggesting that M01G5.1 is involved in supplying directional/positional information. T02C5.1 is featuring a

transmembrane sequence; it is solely expressed in the E blastomere. To our surprise a CRISPR/Cas9 mediated *knock-out* results in aberrant cell positions of the AB derived descendants at the premorphogenetic stage. Cells fail to position correctly especially in the ABa derived regions. The AVD (Average Vector Dissimilarity of cell positions) is with 0.2 very high compared to the 0.08 of WT, while the general migration distance of cells is with 93,2% of WT normal. The normal pattern of cell deaths indicates that cells have their normal identities, but have lost the cell focussing capability depending on positional information. These observations suggest that the genes contribute to directing cells during cell focussing.

438C **Cap-adjacent 2'-O-ribose Methylation is required for Germline Cell Specification in *C. elegans*** Eileen Clemens[1], Sarah Brivio[1], David MacLeod[1], Peter Eiljers[1], Matthias Soller[2], Berndt Müller[1], Jonathan Pettitt[11]Medical sciences, University of Aberdeen, [2]School of biosciences, University of Birmingham

Epigenetic modifications of RNA constitute an important mechanism through which animal gene expression can be regulated, but their individual functions are often poorly understood. One highly conserved RNA epigenetic modification is cap-adjacent 2'-O-ribose methylation, which occurs on the first and second transcribed nucleotides of coding and non-coding RNAs. These modifications were first identified in HeLa cells in the 1970s, but the identities of the key enzymes that catalyse these modifications, the cap-adjacent methyltransferases 1 and 2 (CMTr1 and CMTr2), were revealed only within the last decade. Strikingly, despite its prevalence, we are largely ignorant about the biological role of cap-adjacent 2'-O-ribose methylation.

To understand the molecular and cellular roles of cap-adjacent 2'-O-ribose methylation, we are studying the *C. elegans* CMTr1 and 2 orthologues. Using CRISP engineered strains expressing GFP-tagged versions of both CMTr proteins, we see both proteins in the nuclei of all germline and somatic postembryonic cells. However, during embryogenesis, while CMTr1 is also nuclear, CMTr2 appears to be cytoplasmic. This distinct cellular expression of the two CMTr proteins was consistent with our immunoprecipitation results, which showed different interaction partners for the two proteins. While CMTr2 associated with several cytoplasmic proteins, CMTr1 only showed interactions with the RNA helicase DDX-15, the orthologue of the mammalian DHX15 protein, which co-immunoprecipitated with CMTr1 in mammalian cells. These results indicate that CMTr proteins might have distinct cellular functions in *C. elegans*.

To study those functions, we investigated the effect of CMTr1 and CMTr2 loss-of function mutations. While loss of CMTr2 did not have any effects, CMTr1 loss-of-function mutants failed to produce oocytes, leading to a masculinised germline and associated sterility.

Taken together out data show that CMTr1 has an essential role in germ cell fate determination, but our data also indicate a broader role in somatic cell development. We have recently generated an auxin-inducible degron-tagged version of CMTr1 and are in the process of analysing the molecular defects in animals subject to acute loss of CMTr1 function. Given the conservation of CMTr1 between animals, we expect our results will provide important insights into the impact of cap-adjacent 2'-O-ribose methylation on gene expression and cell fate specification.

439C **Converting molecular- scale torques to embryonic left-right asymmetry** Allan Akandwanaho[1], Teije C Middelkoop-[21]Developmental biology, Institute of Molecular Genetics, [2]Insitute of Molecular Genetics

The vast majority of animals exhibit left-right asymmetric body plans. Left-right asymmetry arises during early embryonic development and is often the result of rotatory movements of cells. Rotational forces, i.e. torques, drive these cellular movements and they originate in the actomyosin cytoskeleton of embryonic cells. In *C. elegans* early embryos, active torque generation facilitates chiral counter-rotatory movements of the actomyosin cortex. In turn, this drives the cell division skews of the ABa/ABp blastomeres and break embryonic left-right symmetry. Active torque generation is facilitated by an actin elongator of the Formin family. At the molecular level, Formins can rotate actin filaments and generate torques. However, how the molecular activity of Formins, which are embedded in a highly cross-linked actomyosin network, can result in chiral rotatory movements remains a mystery. In this project, we aim to understand how torques generated at the molecular level are converted to rotatory movements at the cellular level. To this end, we will combine the strength of *C. elegans* genetics with quantitative live imaging of cortical actomyosin flows in the zygote.

440C **Establishing the role of HBL-1 in the developmental timing of *C. elegans*** Marit van der Does[1], Chiara Azzi[1,2], Adam A. T. Smith[11]Multicellular Systems, Friedrich Miescher Institute for Biomedical Research, [2]babraham Institute

Development of multicellular organisms requires both spatial and temporal control over the formation of different tissues. The well-known transcription factor hunchback in *Drosophila* is important for both the spatial positioning of the anterior/posterior axis, and for the temporal regulation of neuronal cell fates. The *C. elegans* homolog HBL-1 has been shown to be part of the heterochronic pathway, *i.e.*, a gene expression cascade that determines the temporal identity of the different larval stages. However, the exact placement of HBL-1 in the pathway is unknown, and its function as a transcription factor and the identity of

potential targets remain to be established. We show that endogenously tagged HBL-1 is mainly expressed directly after hatch in epidermal cells and ventral nerve cord cells and subsequently downregulated over the first 3 larval stages. To find functional HBL-1 target genes, we performed HBL-1 ChIP-sequencing and RNA sequencing upon depletion of HBL-1. We identify binding sites in the promoters of several heterochronic genes, including that of *lin-29b*, the terminal effector gene that regulates the onset of juvenile-to-adult transition. We demonstrate that HBL-1 depletion and scrambling of its binding sites both lead to precocious *lin-29* expression. These results, and our ongoing investigation of HBL-1-mediated regulation of other heterochronic genes are helping us to understand the placement of HBL-1 in the heterochronic pathway, and therefore how the passage of time is recorded and acted upon.

441C **The SIN-3 HDAC corepressor regulates embryonic development by suppressing the APL-1 expression in *Caenorhabditis elegans*** Mitsuki Ohara, Hamasaki Tasuku, Masahiro Ito, Yukuhiko KubotaDepartment of Bioinformatics, School of Life Sciences, Ritsumeikan University

Class I histone deacetylases (HDACs) are a group of enzymes that demonstrate a remarkable degree of evolutionary conservation from yeast to humans. HDAC corepressor complexes negatively regulate transcription by removing the acetyl group from histones. Corepressor, a component of the HDAC, regulates target gene expression by selectively binding to promoter regions of target genes. HDAC corepressors regulate multiple biological process including cell proliferation, cell migration, and vulval development. However, the regulatory mechanism of the HDAC corepressor complex during embryonic development is not completely comprehended. In our previous study, a deletion mutant of the class I HDAC corepressor, *sin-3(tm1276),* exhibited an embryonic lethal phenotype, suggesting that *sin-3* is required for embryonic development. In addition, we found APL-1 (a *C. elegans* homolog of APP) expression is upregulated in *sin-3* mutant by performing transcriptome analysis.

In this study, we constructed the genomic *bkcSi16[apl-1p::apl-1::gfp]* transgene, which is driven by a 5,100bp promoter and has a 1,940bp 3'UTR. We found that *apl-1p::apl-1::gfp* transgene rescues the larval lethal phenotype of *apl-1(ok1697)* mutant, indicating that *apl-1p::apl-1::gfp* transgene is functional. Furthermore, we checked the transrational APL-1::GFP expression in *sin-3* mutants by calculating the APL-1::GFP reporter signals of *bkcSi16*. These results indicate that the APL-1::GFP reporter signal is significantly increased at 2-cell, comma, and 1.7-fold embryonic stages in the *sin-3* deletion mutant. Moreover, we performed APL-1::GFP-overexpression experiment to understand the effects of APL-1-overexpression during embryonic development. Consequently, an extrachromosomal array containing the *bkcEX24[eft-3p::apl-1::GFP]* transgene, which is driven by an ubiquitously expressing promoter *eft-3p,* exhibits 8.5% embryonic lethality (N=709). In contrast, *bkcEx27[eft-3p::GFP]* exhibits 3.1% embryonic lethality (N=552). These results suggest that *apl-1*-overexpression induces embryonic lethality. Taken together, these results imply that negative regulation of *apl-1* expression is important for precise progression of embryonic development, and *apl-1* is one of the downstream target gene of the SIN-3 HDAC corepressor. To further characterize the importance of SIN-3 HDAC complex, we will analyze the APL-1::GFP expression in the HDAC mutants to identify the histone deacetylase component of SIN-3 HDAC corepressor complex.

442C **Balanced SUMOylation regulates SET-26 "reading" aptness in the germline** Cátia A. Carvalho, Limor BrodayTel Aviv University

SUMOylation is a post translational modification that modulates protein function through the attachment of a SUMO peptide to lysine residues. The reversibility of this linkage is mediated by a family of SUMO-specific proteases – ULP/SENP – conferring dynamics to SUMOylation. We uncovered that ULP-2 is essential for germline viability. Loss of function of ULP-2 results in an accumulation of SUMOylated proteins that progressively induces sterility. Accordingly, germline-specific RNA-seq revealed a broad downregulation of germline enriched genes but also an upregulation of somatic genes, suggestive of ULP-2 modulation of germline cell fate. A Y2H screen revealed a physical interaction between ULP-2 and the H3K4me3 reader SET-26. We show that the two proteins act synergistically to maintain *C. elegans* germline function. Moreover, excessive SUMOylation of SET-26 resulting from ULP-2 loss of function diminished SET-26 ability to bind H3K4me3 marks without affecting its nuclear localization. In addition, using a proteomics approach we observed a decrease of SET-26 ability to bind its interacting partners upon deSUMOylation impairment. Altogether we propose that preservation of physiological levels of SUMOylation on SET-26 is required for its effective reading capacity in sustaining germline function.

443C **Upstream and downstream of *rnt-1* in the seam cells** Yuanhang Jiang, Alison WoollardUniversity of Oxford

Seam cells in *C. elegans* are stem-like, in that they are capable of both self-renewal and differentiation. Asymmetric divisions at each larval stage generate a proliferative posterior daughter cell and an anterior daughter that differentiates, while symmetric divisions at the beginning of L2 (and in additional posterior lineages in males) generate two proliferative daughter cells in order to amplify the number of progenitors.

rnt-1 (the solo *C. elegans* member of the RUNX transcription factor family) has been previously found to be involved in promoting

seam cell proliferation (particularly at the L2 symmetrical division) but the gene regulatory network (GRN) in which RNT-1 operates is not well defined. For example, *rnt-1* (together with its DNA binding partner *bro*-1) acts downstream of *ceh-20* and upstream of *cki-1* and *pop-1* to regulate seam lineage development but the precise nature of these interactions (for example whether they are direct or not) is unclear.

In order to deepen our understanding of the *rnt-1* GRN, we are attempting to identify direct upstream regulators and downstream targets of *rnt-1* using a combination of approaches. For example, we are interrogating a RNT-1 ChIP-seq dataset to identify candidate downstream target genes, followed by functional and expression studies to investigate their potential involvement in RNT-1 mediated seam cell development. To try to identify direct upstream regulators of *rnt-1*, we are investigating conserved non-coding elements (CNEs) in the *rnt-1* regulatory region that are responsible for driving seam cell expression of *rnt-1*, with the ultimate aim of identifying transcription factors binding to such CNEs. We will present our progress in these complementary strands of work.

444C **Endogenous ZIF-1 activity mediates targeted protein degradation in primordial and proliferative germ cells** Aaron ZA Schwartz, Jeremy Nance NYU School of Medicine

Conditional depletion of gene products is the gold standard in deciphering the molecular basis of developmental processes. This can either be done at the mRNA level, via tissue-specific RNAi, or at the protein level, via degron-mediated targeting to the proteosome. Previously, we showed that tissue-specific expression of the endogenous E3 ubiquitin ligase adaptor, ZIF-1, was sufficient to rapidly deplete endogenous proteins tagged with the ZF1 zinc-finger domain of PIE-1. Endogenously, ZIF-1 mediates the degradation of ZF1 domain-containing proteins in the somatic cells of the developing embryo, resulting in the retention of ZF1 domain-containing proteins in the P-lineage. Here, we show that endogenous ZIF-1 becomes active in primordial germ cells (PGCs) shortly after they are born, and remains active in proliferative germ cells throughout larval and adult stages. We show that ZIF-1-mediated depletion of endogenous protein is sufficient to generate null phenotypes in PGCs, and results in the degradation of endogenous ZF1 domain-containing proteins in larval and adult proliferative germ cells. Thus, our findings provide new tools for spatially and temporally eliminating targeted proteins in the germ line.

445C **Oscillatory expression of molting cycle genes is coordinated with pharynx growth in larvae** Timo Louisse[1], Joleen Traets[2], Yvonne Goos[2], Jeroen van Zon[21]Quantitative Developmental Biology, AMOLF, [2]AMOLF

During each of the 4 larval stages in *C. elegans* development the renewal of the cuticle is controlled by the molting cycle. Coupled to the molting cycle is oscillatory gene expression of a large and diverse group of genes. It remains an open question whether these genes are only involved in molting or also play a role in other developmental processes. Interestingly, growth of the pharynx appears to have a cyclical character, indicating a potential link with the molting cycle. Here, we studied the interplay between pharynx-specific oscillatory genes and the growth of the pharynx. Using RNA-sequencing, single molecule FISH (smFISH) experiments and time-lapse imaging of fluorescently tagged proteins, we identified a group of oscillatory genes that are expressed exclusively in the pharynx and peak at the early intermolt. These genes include *myo-1*, *myo-2* and *marg-1*, genes that likely play a structural role in pharynx muscle. Moreover, measuring the size of the pharynx using time-lapse imaging during larval development revealed that the rate of pharyngeal growth was not constant, but, like expression of these genes, peaked at the early intermolt. We then used starvation-induced developmental arrest to perturb pharynx growth and examine the impact on oscillatory gene expression. In L1-arrested animals, both body and pharynx growth ceased simultaneously. In L3- or L4-arrested animals, however, pharynx growth continued for hours after the arrest of body growth, until it reached a size corresponding to that seen at the time of the subsequent ecdysis during normal development. Consistently, in fully arrested animals we found that expression of oscillatory pharynx genes was halted at a phase corresponding to ecdysis. Moreover, using time-lapse imaging we observed that continued pharynx growth after starvation-induced arrest of body growth is accompanied by one aberrant cycle of oscillatory gene expression. Finally, we examine transcriptional regulation of *myo-2* by PEB-1, a transcription factor that oscillates out of phase with *myo-2*. Overall, the observed coordination between the pharynx growth and expression oscillations of pharynx genes, both during normal development and following arrest, suggest that oscillating expression of these genes is functionally linked with organ growth.

446C **Establishing the polarity of intracellular lumenogenesis** Lauren Meyer, Jeremy NanceNYU Grossman School of Medicine

Epithelial tubes are a major component of most organs in multicellular organisms, thus tubulogenesis and the maintenance of epithelial tubes is required for organ function and multicellular life. The C. elegans excretory cell provides a model for investigating the smallest of epithelial tubes: unicellular, seamless tubes. In the excretory cell, lumenogenesis occurs through polarized vesicle trafficking that extends a nascent lumen membrane through the center of the cell creating a hollow, intracellular lumen. This study addresses the nature of the nascent lumen at the time of apical vs basolateral polarity establishment, as well as the

molecular mechanisms that may orient the polarity establishment process. Here, I demonstrate a method for characterizing the inception of lumen formation through live imaging. Additionally, I describe the results of a forward genetic screen that suggest that the transmembrane receptor, integrin, plays a major role in regulating excretory cell lumen development.

447C **MSc Research project_ The understanding of body wall muscle cells' fusion by ectopics fusion of the fusogen EFF-1 and his effect in PVD's arborization Sharon Fitoussi_The technion** Sharon FitoussiBiology, The technion

Fusogens are molecules or proteins that mediate the fusion of membranes. In *C. elegans,* one such fusogen is EFF-1 (Epithelial Fusion Failure-1) which is a transmembrane protein that plays a critical role in the fusion of cells. Studies have shown that EFF-1 drives fusion of heterologous cells into multinucleate syncytia. Furthermore, such cell-cell fusion events requires EFF-1 in both fusing cells(Avinoam et al. 2011; Meledin et al. 2020; Podbilewicz et al. 2006).

The body wall muscle cells (BWMs) are a type of muscle cell that make up the body wall and play a crucial role in the worm's movement and posture. These cells are mononuclear and organized into a network of muscles that run parallel to the body axis separated into four different quadrants of cells. All these cells coming from different lineages allowing for precise and coordinated movement(Altun, Z.F. and Hall 2009b). All these cells are coming from different time point of cell division, which creates different lineages of cells, allowing a precise and coordinated movement. Additionally, these cells are, as other muscles in the nematode, under the control of the nervous system, allowing for response to changes in the environment(Moerman and Fire 1997).

PVD neurons, are one of the 302 nematode's neurons responsible for detecting vibrations, low temperatures, propioception and strong mechanosensory stimuli in the nematode's environment(Inberg et al. 2019; Oren-Suissa et al. 2010). Parts of the highly arborized dendritic branching of the PVD have been showed to be developed exactly between the BWMs and the epidermis(Zou et al. 2016).

In recent research in our lab, in order to determine the effect of EFF-1 expression in cell fusion of BWMs, we chose to express EFF-1 ectopically in BWMs cells, which typically do not fuse and do not form multinucleated myofibers(Altun, Z.F. and Hall 2009a). We expressed ectopically with an extra chromosomal array EFF-1 under a muscle specific myosin-3 promoter (*myo-3p*::EFF-1) in BWMs specific for the muscle function. The results showed that EFF-1 expression in BWMs can induce their ectopic fusion, changing their structure. These nematodes with BWMs fused become uncoordinated and dumpy (Unc and Dpy respectively)(Meledin et al. 2020). Suggesting that if a fusogen such as EFF-1 is expressed in muscle cells, their fusion can occur. We found a positive correlation between the extra-chromosomal EFF-1 array and BWMs fusion. We also found that the number of fused cells is related to the length of each worm respectively. Even more phenotypes were found during our investigation. These findings expand our knowledge on the regulation of muscle development and function and provide valuable insights into the underlying mechanisms of EFF-1-mediated BWM cell fusion. Next experiment will give us more knowledge about the impact of this ectopic fusion on PVD's arborization.

448C **Genetic evidence that the Pumilio-family proteins PUF-3 and PUF-11 repress SPN-4 translation in oocytes to prevent premature Ccr4-Not-mediated maternal RNA destabilization** Erika Tsukamoto, Micah Gearhart, Caroline Spike, David Greenstein Genetics, Cell Biology, and Development, University of Minnesota

Purified LIN-41-containing ribonucleoprotein (RNP) complexes contain several different RNA-binding proteins, including the nearly identical and functionally redundant Pumilio-family RNA-binding proteins PUF-3 and PUF-11 (Tsukamoto et al. 2017). LIN-41 and PUF-3/11 promote the 3'UTR-mediated translational repression of the Rbfox-related RNA-binding protein SPN-4 in oocytes (Hubstenberger et al., 2012; Tsukamoto et al., 2017). Interestingly, the maternal-effect embryonic lethality caused by null mutations in *puf-3/11* is partially suppressed by select mutations in *spn-4*: suppression can be observed in animals homozygous for the GFP-tagged allele *spn-4(tn1699)* or heterozygous for the maternal-effect-lethal *spn-4(tm291)* deletion allele. These observations suggest that SPN-4 over-expression in oocytes contributes to *puf-3/11* embryonic lethality. To understand this more completely, we screened 28,498 EMS-mutagenized haploid genomes for new suppressors of *puf-3/11* lethality and identified 24 strains where *puf-3/11* lethality was suppressed enough to permit backcrossing. Whole genome sequencing was used to identify candidate suppressor mutants in each strain. As expected, we identified a new allele of *spn-4*; one of the suppressed strains was heterozygous for a maternal-effect-lethal allele of *spn-4* with a premature stop codon. More intriguingly, nine of the suppressed strains carried independent mutations in two different genes that encode essential subunits of the Ccr4-Not mRNA deadenylase complex in *C. elegans*: *let-711*/Not1 (8 strains) and *ccf-1*/Caf1 (1 strain). Most of these mutations were heterozygous in the suppressed strains and likely to be strong loss-of-function alleles. We have confirmed that other, independently-generated, larval-lethal alleles of *let-711* dominantly suppress *puf-3/11* maternal-effect embryonic lethality and have preliminary results that suggest this will also be true for similar alleles of *ccf-1*. Based on these and other observations (see the abstract by Spike et al., this meeting), we propose that the SPN-4 RNA-binding protein plays an important role in targeting specific mRNAs for deadenylation and decay during the oocyte-to-embryo transition in *C. elegans*. According to this model, in *puf-3/11* null mutants, SPN-4 is

precociously expressed in proximal oocytes and causes premature clearance of maternal mRNAs with which it associates. Tests of this model are underway.

449C **Temporal Requirement for Insulin Signaling in Developmental Progression During Embryogenesis** Isabella C van der Weide, Bruce Wightman Biology, Muhlenberg College

DAF-2 is the main insulin receptor in *C. elegans*, to which the rest of the insulin signaling response pathway responds. The pathway exhibits temperature-dependence: *daf-2(e1370)* mutant animals develop normally when grown at 15°C, but enter dauer when grown at 25°C. Addition of a mutation in *fax-1*, a gene encoding a nuclear hormone receptor involved in neuron development, leads to earlier developmental arrest in animals grown at 25°C. The vast majority of *daf-2(e1370); fax-1(gm83)* embryos grown at 25° C arrest just before or just after hatching in a quiescent, non-pumping state, a phenotype we call peri-hatching arrest. We hypothesized that there may be some insulin signaling-dependent developmental moment during embryogenesis that double mutant animals are able to progress through at 15°C, but not at 25°C. To test this, I performed a series of temperature upshift experiments during mid- to late- embryogenesis. I found that the ability of *daf-2(e1370); fax-1(gm83)* animals to develop past the first larval stage was dependent on reducing insulin signaling during mid-embryogenesis. In populations upshifted at 4 hours after egg-lay, 98% of animals (list approximate stage) exhibited peri-hatching arrest, compared to only 19% of animals that were upshifted at 10 hours. These data suggest that insulin-signaling for developmental progression at hatching depends on a function in mid-embryogenesis, or the progressive accumulation of insulin signaling over that period, rather than at the time of hatching. These results are also consistent with the progressive decrease in embryonic movement over the second half of embryogenesis in *daf-2; fax-1* double mutants.

450C **The role of the *let-7/lin-41* pathway in the neuronal maturation of *Caenorhabditis elegans* hermaphrodites** Craig Peters, Dalton Patterson, Guihua Zhou, HaoSheng SunCell, Developmental, and Integrative Biology, University of Alabama at Birmingham

Across the animal kingdom, the post-mitotic neurons that make up our nervous system do not change much in number between the adolescence/pubertal periods to adulthood. These neurons undergo extensive maturational changes from their molecular composition to cellular properties, ultimately leading to behavioral maturation. The underlying molecular mechanisms that control these maturational changes are not well understood. The regulatory pathway involving the evolutionarily conserved *let-7* microRNA and its downstream target, the RNA-binding protein *lin-41*, is of particular interest because it has been previously identified to play a role in the temporal progression of mitotic cell types (i.e., epithelial, muscle, etc.) during *C. elegans* L4 (analogous to pubertal periods in other animals) to adult transition. This pathway has also been shown to control the maturation of the male *C. elegans* nervous system during the L4 to adult transition by additional regulation of the *LIN-29* transcription factor. The examination of this pathway in the hermaphrodite nervous system, however, has been limited to a handful of midbody neurons that only express *LIN-29*. In this study, we aim to understand the role of *lin-41* and *let-7* in the L4 to adult maturation of the entire hermaphrodite nervous system. Using transgenic fluorescent reporters, the expression patterns of *lin-41* and *let-7* across post-embryonic development were characterized in the entire nervous system, including the AIB interneuron, which plays a crucial role in controlling forward/backward locomotion. To examine the functional role of this pathway, we examined forward/backward locomotion that demonstrates robust maturational changes during the L4 to adult transition in both wildtype and mutant animals. We found that downregulation of *lin-41* across post-embryonic development was important in the maturation of the AIB interneuron and forward/backward locomotion: forced expression of *lin-41*, specifically in the adult AIB neuron, was sufficient to rejuvenize behavioral parameters to resemble that of L4 animals. Next, we are identifying the molecular targets by which *let-7/lin-41* control AIB maturation as well as examining their role in the maturation of other neurons using cell-type-specific molecular profiling. Understanding the mechanism by which *let-7/lin-41* controls neuronal maturation between the crucial transition from adolescence to adulthood will allow us to better understand neurodevelopmental processes and give insights into how dysregulation of these processes lead to disorders associated with pubertal timing.

451C **A bacterial genetic screen to identify how diet regulates *C. elegans* germline stem cells** Katherine Norton, Julia Burnett, E. Jane Albert Hubbard Cell Biology, NYU Grossman School of Medicine

Stem cells are capable of self-renewal and of producing cells that differentiate, and they can also respond to changes in an organism's environment such as the quantity and quality of its diet. In many stem cell systems, the stem vs. differentiated cell fate decision is controlled via interaction with a stem cell niche. In the *C. elegans* germline stem cell system, ligands for the Notch receptor are expressed in the niche, the distal tip cell (DTC), which activate a Notch receptor (GLP-1) in neighboring germ cells to maintain stem cell fate.

Previously, our lab found that DAF-7 TGFß, the neuronal expression of which is modulated by diet, acts through its receptor DAF-1 on the DTC such that diet alters the expression of a ligand for Notch, modulating the number of germline stem/progenitor cells

that accrue during larval stages (Dalfó et al., 2012 *Current Biology*; Pekar et al., 2017 *Development*). However, we do not know which components of the *C. elegans* diet act on this TGFß "neuron-to-niche" axis to regulate germline stem cell fate.

To identify relevant dietary components, our first strategy (Keio screen v.1) was to screen the Keio *E. coli* mutant library for *E. coli* mutants that when fed to worms, impaired fertility in a sensitized mutant background, mimicking the loss of *daf-7* in this background. Although the approach identified several candidates, they did not affect the stem cell pool. Nonetheless, they offered insight into the interaction between the vertebrate microbiome and a parasitic nematode (Venzon et al., 2022 *Cell Host and Microbe*).

In Keio screen v.2, we are looking for *E. coli* mutants that, when fed to worms, affect a key read-out of the neuron-to-niche axis in the DTC. In high through-put 96-well liquid format, we track a DTC marker that is visible in wild-type worms but is not visible when the DAF-7 TGFß axis is perturbed. Counts of both worms and DTC detection are automated, and we have developed a pipeline for scoring that considers the density of the bacteria and the number of scorable worms. Secondary screening tests candidate bacterial mutants for robust reproducibility. So far, 525 mutants from 6 of the 48 Keio library plates have been scored. Of these, 62 were identified as candidates with our updated pipeline, all of which have failed in the secondary screening process, suggesting our screening strategy is sufficiently rigorous. Primary and secondary screening of the remaining Keio library plates are in progress.

452C **Elucidating the Localization and Functions of SPD-1 during Chromosome Segregation in *Caenorhabditis elegans* Sperm Meiosis** Cuc Huynh[1], Diana Chu[21]Department of Biology, San Francisco State University, [2]San Francisco State University

While male infertility brings significant challenges to human reproduction, little is known about the mechanisms that drive male meiotic chromosome segregation required for efficiently generating healthy sperm. To ensure accurate chromosome segregation during mitosis and oocyte meiosis, microtubules are organized into a central spindle structure that forms between the separating chromosomes. CLS-2 initiates the central spindle assembly and recruits SPD-1, a microtubule bundling protein, for microtubule elongation. Interestingly, our studies in Caenorhabditis elegans nematodes have shown that a lagging unpaired X chromosome seems to minimize the formation of the central spindle in sperm meiosis. It is unclear whether sperm meiosis relies on the central spindle for proper chromosome segregation. Utilizing immunohistochemistry and live confocal microscopy, I aim to determine the localization patterns and functions of SPD-1 during anaphase of sperm meiosis. Results show that SPD-1 localizes at the midzone region despite the lack of a classic central spindle during sperm meiosis in both the presence or absence of the lagging unpaired X chromosome. This finding suggests that SPD-1 may function independently from CLS-2 during sperm meiosis because CLS-2 is known to remain chromosome-associated when X chromosomes do not lag. This further reveals the unique role of SPD-1 in maintaining microtubules stability for proper chromosome segregation during sperm meiosis. Taken together, this body of work will elucidate why chromosomes sometimes fail to segregate during meiosis in sperm cells and may eventually lead to new strategies for combating male infertility.

453C **Genetic mechanisms that restrict dendritic branching** Christopher J Salazar[1], Carlos A Diaz-Balzac[1], Leo Tang[1], Jenna Freund[1], Yu Wang[2], Barth D Grant[2], Hannes E Buelow[11]Albert Einstein College of Medicine, [2]Rutgers University

Dendrite development depends on extracellular and intracellular cues to ensure proper structure and function. However, the regulatory mechanisms of dendrite development remain incompletely understood. To better understand dendrite development, we are utilizing the PVD somatosensory neuron with its highly stereotyped 'menorah'-like dendrites. The Menorin genetic pathway consists of several factors that function from different tissues to promote PVD dendrite development. While progress has been made in understanding factors that promote formation of dendritic branches, less is known about factors that restrict branching. During genetic screens, we have identified two loci that are important to restrict branching of PVD dendrites. One locus encodes a putative, uncharacterized Rab-related GTPase, which we name *rabr-1*. In *rabr-1* mutants, the number of PVD branching points increased into adulthood, suggesting that the normal function of *rabr-1* is to restrict dendritic branching into adulthood. A transcriptional reporter for *rabr-1* is expressed in the epidermis from early embryonic through adult stages and transgenic expression of a *rabr-1* cDNA in the epidermis rescues the mutant phenotype. This suggests that *rabr-1* functions non-autonomously from the epidermis to restrict PVD branching. A rescuing N-terminal tagRFP::RABR-1 fusion displays intracellular, perinuclear staining. Colocalization analyses reveal that tagRFP::RABR-1 staining is correlated with late endosomes and autophagosomes, and anticorrelated with lysosomes, indicating that tagRFP::RABR-1 may be localized to amphisomes. Genetic analyses place *rabr-1* in the Menorin pathway suggesting that *rabr-1* may be important for the regulation of epidermal factors of the Menorin pathway. We are currently in the process of identifying the factors that may be regulated by *rabr-1.* The second locus to restrict branching is represented by a single allele, *dz272*, which also displays a hyperbranching phenotype in PVD dendrites that is similar to mutants in *rabr-1. dz272* complements *rabr-1* and maps to a different chromosome suggesting that we have uncovered a second gene important for the control of dendrite branching. We will report on our progress in studying these

two loci and their roles in regulating PVD dendrite morphogenesis.

454C **LIN-28 as a conserved factor controlling the juvenile-to-adult transition** Jana Brunner, Anca Walczak, Helge Grosshans Friedrich Miescher Institute for Biomedical Research

The transition from juvenile to sexually mature state, known in mammals as puberty, is a fundamental feature of animal development. In the *C. elegans*, this transition occurs after four larval stages under the control of the heterochronic pathway, an inhibitory gene cascade consisting of two parallel arms. We aim to understand how the activities of two arms are coordinated to ensure synchronous development of different body parts and, thereby, a smooth entry into adulthood. We hypothesize that the coordinating factor is the conserved RNA-binding protein LIN-28. To uncover the mechanisms by which LIN-28 orchestrates development, we aim to characterize the molecular interaction with its two established targets, *lin-46* mRNA and *let-7* miRNA. We mutated the putative LIN-28 binding site in *lin-46* 5'UTR and *let7* primary transcript and analyzed the effect on their expression. While the mutation had no effect on mature *let-7* miRNA levels, the analogous mutation introduced in *lin-46* transcript resulted in precocious accumulation of LIN-46 protein. To further understand how LIN-28 represses its targets, we performed an IP-MS experiment to identify its potential co-factors. Among the interacting partners of LIN-28 we identified some of the RNA-binding proteins previously shown to interact with the mammalian LIN28, such as SSB-1 (homolog of SSB), LARP-1 (LARP1) or FIB-1 (FBL). Currently we are screening the interacting partners for causing developmental phenotypes upon RNAimediated knock-down to further assess the functional relevance of their interaction with LIN-28.

455C **Investigating the temporal gene regulation of grinder formation** Sage Aviles, Matthew D Nelson Biology, Saint Joseph's University

The pharyngeal grinder is an essential complex extracellular structure whose dissolution and assembly occurs during each larval transition (i.e., lethargus and ecdysis). *lin-42* encodes the PEROID homolog and functions as a developmental timer; how grinder developmental is temporally controlled is not well understood. We used transmission electron microscopy (TEM) to examine the ultrastructural formation of the grinder, which involves pharyngeal trans-differentiation from a contractile into a secretory cell (Sparacio et al. 2020). A rapid increase in transcription leads to the upregulation of abu/pqn paralog group (APPG) genes, termed *pqn* and *abu*. Many of these are expressed in pharyngeal muscle and localize to the pharyngeal cuticle and grinder (George-Raizen et al. 2014). We aim to better understand how *lin-42* regulates the timing of APPG gene expression and potentially the localization of their gene products. To do this, we are quantifying the timing of APPG gene expression in both wild-type and *lin-42* mutant animals. Additionally, we are using TEM and ascorbate peroxidase 2 (APEX2)-tagged APPG transgenic animals, to characterize the timing of the ultrastructural localization of these key grinder components.

456C **Single cell atlases in diverse nematode species** Manuela Rebecka R Kieninger[1], Mark Blaxter[21]Tree of Life/ Blaxter faculty, Wellcome Sanger Institute, [2]Tree of Life, Wellcome Sanger Institute

Understanding of the molecular mechanisms of how cells adopt their terminal cell identity is central to developmental biology. In *Caenorhabditis elegans* changes of cellular state are dependent on the cell lineage, cell positioning, and the cell's transcriptional program. Single cell sequencing has made it possible to define novel cell states and re-define molecular signatures of cell types beyond the known marker genes expressed or explored in forward and reverse genetic screens. Development evolves, like any other trait, but most studies on cell type differentiation have used a single species. Comparative studies involving more than one species are scarce and their resolution is generally limited to single-system or whole-organism transcriptome data.

We are exploring the transcriptomic variation across the phylum Nematoda at the single cell level. Advances in sequencing and microfluidic technologies allow high-throughput cell processing to study heterogeneity and classify single cells. Packer et al. showed that single-cell sequencing data of *C. elegans* can track the development of cell identity during embryogenesis. Starting with *C. elegans* as a reference dataset, we will generate single-cell transcriptome data across various *Caenorhabditis* species, and then expand to other rhabditine nematodes to ask how homologous gene expression has evolved through diversification and within homologous tissues. We culture mixed stage embryos for nuclei isolation and prepare single-nucleus libraries with the 10xGenomics system.

In summary, we will provide quantitative and high-resolution datasets for the transcriptome of various nematode species during embryogenesis. They are the fundamental requirement for studying the evolution of cell differentiation programs and yet might be equally valuable for understanding how novel cell types originate.

457V **GLD-1 and GLD-2 Physically Interact with the GLP-1/Notch Receptor intracellular domain** Xue Han[1], Dan Zhang[1], Ryan Smit[1], Priyanka Grewal[1], Tim Schedl[2], Dave Hansen[11]University of Calgary, [2]Washington University School of Medicine

Reproductive fitness requires a balance between stem cell self-renewal and differentiation. In the *C. elegans* germline, this

balance is largely controlled by GLP-1/Notch signaling. In the distal region of the germline, high GLP-1/Notch levels promote the proliferative fate. GLP-1 signaling levels decrease proximally, allowing the GLD-1/NOS-3, GLD-2/GLD-3 and SCF^{PROM-1} redundant pathways to be activated, directing cells to enter meiosis/differentiation. Therefore, a stem cell's decision to self-renew or differentiate is governed by the establishment of opposing gradients of GLP-1 signaling and the GLD-1/GLD-2/SCF^{PROM-1} pathways along the distal-proximal axis of the germline. Previously, GLP-1 has been shown to indirectly repress the translation of *gld-1* and *gld-3*. However, recent data from our lab suggests a potential new means of regulation between GLP-1 and GLD-1/GLD-2 that could result in the repression of their normal regulatory roles. We demonstrate that the GLP-1 intracellular domain (intra) physically interacts with both GLD-1 and GLD-2, suggesting another potential mode of regulation. To test this, we determined the protein regions required for protein interactions using a yeast two-hybrid system. Results obtained thus far indicate that GLP-1(intra) interacts with the N-terminal regions of GLD-1 and GLD-2. More work is underway to identify key amino acids in GLD-1 and GLD-2 for interaction with GLP-1. We are also studying how this protein interaction may negatively regulate GLP-1/Notch or the GLD-1/GLD-2 pathways. One approach we are using is to analyze whether ectopic expression of the N termini of GLD-1/2 domains results in a change in progenitor zone length in three *glp-1* mutants that are sensitized genetic backgrounds. This research will contribute to our understanding of the precise regulatory mechanisms governing the balance between stem cell proliferation and differentiation.

458V **DAF-18/PTEN and the maintenance of VPC quiescence in dauer larvae** Alexandra Ketcham[1], Catherine O'Keeffe[21]Biological Sciences, Columbia University, [2]Cell Biology, New York University School of Medicine

When conditions are unfavorable for growth or reproduction, *C. elegans* enters dauer diapause, a reversible state of developmental quiescence. Both dauer formation and exit entail vast coordinated changes throughout the body. Dauer entry interrupts vulval induction, and a reprogramming-like event preserves the quiescence and multipotency of Vulval Precursor Cells (VPCs) so that vulval development can proceed during recovery if conditions improve (Euling & Ambros 1996; Karp & Greenwald 2013). During vulval induction, LIN-3/EGF from the gonad activates EGFR in the nearest VPC, P6.p, and the three central VPCs adopt vulval fates. The three outer VPCs divide and their daughters fuse with hyp7 (the non-vulval 3° fate). In dauer larvae, the response to EGF is attenuated; the VPCs remain quiescent with L2-like and unique dauer features (O›Keeffe and Greenwald 2022).

In dauers, *daf-16/*FoxO acts cell autonomously: loss of *daf-16* in VPCs restores vulval induction (Karp & Greenwald 2013; O›Keeffe & Greenwald 2022). By contrast, we found that in dauers lacking DAF-18/PTEN, VPCs often divide and take on the 3° fate. And, although post-dauer *daf-16(0)* adults have an induced, albeit abnormal, vulva (Karp & Greenwald 2013), post-dauer *daf-18(0)* adults are often vulvaless.

daf-18 has a complex nonautonomous focus within the somatic gonad that maintains quiescence of the somatic gonad and germline (Tenen & Greenwald 2019). To identify the cellular focus of *daf-18* for maintaining quiescence of dauer VPCs, we used Cre/lox to remove *daf-18* tissue-specifically. Loss of DAF-18 from the VPCs did not cause them to divide. We also removed DAF-18/PTEN from several other individual tissues, including the somatic gonad, and did not observe highly penetrant loss of VPC quiescence. However, we do observe highly penetrant loss of VPC quiescence when we remove DAF-18/PTEN from certain groups of tissues by combining Cre drivers. Thus, our results suggest that *daf-18* also has a complex nonautonomous focus to maintain quiescence of VPCs. We are currently using different combinations of more specific Cre drivers and tissue-specific rescue constructs to define the complex focus further.

The striking nonautonomy in both gonadal and nongonadal soma suggests that *daf-18* is required for intercellular communication or potentially can move itself. We are performing a targeted RNAi screen for factors that may illuminate this process.

459V **GLD-1 function is regulated by RACK-1 in the germline of *C. elegans*** Kaitlin Chan[1], Kara Vanden Broek[1], Emily Osterli[2], Sadaf Sangari[1], Megan Olson[1], Ekaterina Voronina[2], David Hansen[11]University of Calgary, [2]University of Montana

GLD-1 is a translational repressor involved in regulating a variety of germline functions, including oocyte development, hermaphrodite spermatogenesis, and facilitating entry and progression through meiosis. GLD-1 accumulates in the cytoplasm of cells in a distinctive pattern, which has been shown to be important in regulating its activity. GLD-1 levels are low distally, but gradually increase in more proximal cells, until reaching maximum levels near the transition zone. Levels decrease rapidly in the loop region. This pattern of GLD-1 expression has been shown to be regulated both transcriptionally and translationally. Here we describe an additional level of GLD-1 regulation, which is achieved through its subcellular localization. While cytoplasmic in wildtype animals, GLD-1 mislocalizes to P granules and its expression levels are reduced in the absence of *rack-1*. RACK-1 is a highly conserved scaffold protein that can bind, anchor, and shuttle a variety of protein partners. It is known to be involved in pathways including ribosome assembly, miRISC recruitment to ribosomes, and cell cycle regulation. Here we demonstrate that RACK-1 binds to GLD-1, affecting its subcellular localization. Using three different methods, we found that RACK-1 and GLD-1 physically interact. First, RACK-1 co-immunoprecipitates with GLD-1 from worm lysates, and *vice versa*. Second, bacterially

expressed RACK-1 and GLD-1 co-purify. Finally, GLD-1 and RACK-1 were shown to be in close proximity using proximity ligation assays (PLAs), which demonstrate that the interaction between RACK-1 and GLD-1 occurs in regions of the gonad consistent with *rack-1(0)* and *gld-1(0)* mutant phenotypes. Loss of *rack-1* partially suppresses the Glp phenotype in a *fbf-1(0) fbf-2(0)* mutant background, phenocopying the suppression observed when *gld-1* activity is partially reduced (*gld-1(het)*), suggesting that the mislocalization of GLD-1 partially reduces GLD-1 function. Altogether, this data supports our model that RACK-1 is important for regulating GLD-1 function by controlling its subcellular localization. RACK-1 may bind GLD-1 in the cytoplasm to prevent it from going to P granules. If RACK-1 is absent, GLD-1 does not function properly and becomes mislocalized to P granules. This uncovers a new mechanism by which GLD-1 activity is regulated.

460V **Exploring the role of C. elegans furin proteases in the cleavage of ZP proteins** Chelsea Darwin, Helen Schmidt, Susanna Birnbaum, Meera Sundaram Genetics, University of Pennsylvania

Zona Pellucida (ZP) domain proteins are a family of glycoproteins first characterized in the coating of mammalian egg cells. In *C. elegans*, these proteins are important structural components of the pre-cuticle apical extracellular matrix (aECM). Improper localization of these proteins to the matrix during development results in structural defects. Many ZP proteins are cleaved at a dibasic site that resembles the consensus for furin proteases, and this cleavage has been shown to be essential for function and correct localization to the matrix. However, the protease(s) responsible for cleavage have not been identified.

C. elegans has 4 furin proteases: BLI-4, KPC-1, EGL-3, and AEX-5. In collaboration with the lab of Andrew Chisholm (UCSD), we recently showed that BLI-4 promotes collagen secretion and cuticle assembly in the embryo. However, despite their dramatic cuticle defects, *bli-4* null mutants still had normal localization of ZP proteins in the embryo pre-cuticle.

Here we use the developing *C. elegans* vulva as a model to investigate the *C. elegans* furins and their relationship to the ZP proteins LET-653 and NOAH-1. Of the four furins, KPC-1 and BLI-4 have been positively identified as expressed in vulval cells and are therefore likely candidates for this proteolytic cleavage. Preliminary data from *kpc-1* null mutants and *bli-4* isoform specific knockouts suggest neither affect the localization of either ZP protein. However, *bli-4* null mutations are lethal, so it has proven challenging to visualize a complete gene knockout in the vulva. Additionally, it is possible these furins may compensate for one another and having a functional copy of either one is sufficient. Future experiments will use *bli-4* RNAi on a vulva- specific RNAi strain, which will allow us to knock down *bli-4* only in vulval cells and avoid lethality. We also plan to use double mutants and RNAi to look for redundancy among the different furin genes. Finally, if necessary, we will extend our screening to other types of proteases.

461V **Roles for fate specifying transcription factors in collective cell migrations and fate transformations in *C. elegans* embryogenesis** Prativa Amom[1], Breana D. Anderson[1], Tushar H. Ganjawala[1], Erin Hsiao[1], Jorin T. Hanson[1], Radmehr Molaei[1], Amanda L Zacharias[1,21]Developmental Biology, Cincinnati Children's, [2]Pediatrics, University of Cincinnati School of Medicine

In vertebrates, cell fate specification is directly linked with collective cell migrations. In *C. elegans*, the role for lineage determining factors is established in gastrulation, but the role for fate specifying transcription factors in gastrulation and other collective cell movements including ventral cleft closure remained unknown until recently. We previously evaluated the roles of the transcription factors (TFs) that specify neuronal, muscle, skin, and pharyngeal fates to determine their roles in collective cell movements. We found that the skin TFs *elt-1* and *nhr-25* are required to promote mesoderm gastrulation, pharynx organization, and ventral cleft closure, while two muscle fate TFs *hlh-1* and *unc-120* also promote ventral cleft closure. To determine whether fate transformations played a role in disrupting collective cell movements in these embryos, we examined the expression of various cell fate markers in mutant embryos. In particular, we wanted to test the hypothesis that cells would express the fate markers of "sister" lineages, since their shared history suggests that they might have the factors necessary to activate the same genes.

We found that in *nhr-25(jm2389)* mutant embryos, some Caa and Cpa daughter cells that would normally adopt hypodermal fates activate an unc-120 promoter reporter, indicating they have activated the muscle fate of their Cap and Cpp cousin cells. This is consistent with previous reports that utilized RNAi knockdown and microarray to evaluate gene expression. Conversely, in *hlh-1(cc561)* mutant embryos treated with RNAi against *unc-120*, the MSap and MSpp daughters that normally produce muscle do not activate a PHA-4::GFP fosmid reporter, a marker of pharyngeal fate, which is expressed by their MSaa and MSpa cousin cells. These results suggest that different lineages have evolved distinct mechanisms for fate specification. While our findings are consistent with previous reports that the TFs specifying muscle and skin fates are mutally antagonistic in the C lineage, they also suggest that the mechanism(s) for establishing expression of muscle and pharyngeal fate TFs in distinct sister lineages of MS may be more complex.

462V **Vitamin B12 levels affect Ephrin and Netrin signaling to create a gene-environment interaction in *C. elegans* embryonic development** Erin Hsiao[1], Prativa Amom[1], Radmehr Molaei[1], Amanda L Zacharias[1,21]Cincinnati Children's, [2]Pe-

diatrics, University of Cincinnati School of Medicine

In the natural environment, *C. elegans* embryos develop robustly despite daily temperature shifts, different bacterial food sources, and other variable environmental conditions. In standard laboratory conditions, the worms receive a single food source, *E. coli*, resulting in partial deficiency of vitamin B12. In humans, maternal deficiency in vitamin B12 can result in persistent neurological defects, including neural tube closure defects. Vitamin B12 levels also interact with folic acid levels, another nutrient linked to neural tube closure, in the methionine/SAM cycle.

We investigated the impact of vitamin supplementation on mutants carrying mutations in the ephrin receptor gene, *vab-1*. Over half of *vab-1* mutant embryos fail to hatch due to defects in the closure of the ventral gastrulation cleft, a tissue fusion event similar to mammalian neural tube closure or palatal fusion. We found vitamin B12 supplementation partially rescues the embryonic lethality of seven different *vab-1* mutant strains, but does not affect neuronal and head phenotypes. The average embryonic hatch rate increased from 40% to 63% for null alleles across multiple days of egg laying. Feeding *Comamonas*, a bacteria which produces vitamin B12, results in a similar level of embryonic rescue. Vitamin B12 supplementation also partially rescued mutants carrying mutations in *vab-2, eph-2, eph-4* (all ephrin ligands), and *sax-3*/Robo receptor, but not mutations in other developmental pathways. Folic acid supplementation also partially rescues the lethality of *vab-1* mutants, but does not further increase embryo survival when added with vitamin B12, indicating they act in the same pathway. Since *unc-6*/netrin is expressed in the cells that close the cleft we investigated the mutant *ev400*, which we found has embryonic lethality of 22% and can be almost completely rescued by vitamin B12 supplementation.

We are currently investigating how ventral cleft closure in mutant embryos is affected by vitamin B12 supplementation using time lapse imaging and whether increased histone or protein methylation triggered by vitamin B12 supplementation might affect levels of ephrin and netrin pathway genes. Our findings indicate that *C. elegans* has strong potential to investigate gene-environment interactions in basic developmental processes.

463V **Ral protein but not Ral signaling activity is required for exocyst function** You Wu, David ReinerTexas A&M IBT

The Ras small GTPase is the most mutated oncoprotein. Signaling through the RalGEF-Ral effector remains poorly understood. Ral (Ras-like) is a small GTPase related to Ras. Ral performs two general functions: it both uses the exocyst complex as a signaling intermediary and performs essential activities to regulate exocytic functions of the exocyst. Yet the large majority of studies exploring exocyst functions have been performed in yeast, which does not encode Ral. These features of Ral biology preclude conventional biochemical bootstrapping to identify signal transduction components of Ral downstream of the exocyst. Delineating the mysterious functions of Ral in signaling vs. Ral-dependent functions of the exocyst are important for therapeutic targeting of oncogenic Ras and understanding the cell biological functions of the exocyst complex.

We are investigating roles of Ral in control of exocyst functions during development. First, we have established an animal model for exocyst function using *C. elegans* and determined whether Ral is needed for exocyst function. Second, we have genetically separated signaling functions of Ral vs. function of Ral as component of the metazoan exocyst complex.

We used defects in animal growth and PVD neuron arborization as read-outs for function of the exocyst. We found Ral is needed for the exocyst function, as Ral deletion aggravated phenotypes of exocyst component deletions in both assays. In contrast, defects in the Ras>RalGEF>Ral signaling cascade, which uses the exocyst as a signaling intermediary, do not alter exocyst functions of Ral; therefore, the exocyst does not depend on Ral signaling, contradicting much earlier findings in mammalian cell culture.

We will continue to test the contributions of Ral to exocyst-dependent development via tags of relevant endogenous proteins, missense mutations that further uncouple functions, and using biochemical tools and confocal imaging to measure the physical interaction and colocalization of tagged Ral and exocyst components.

All previous studies about Ral in metazoans deleted/depleted both Ral signaling- and exocyst-related functions. Consequently, we still do not know the role of Ras>RalGEF>Ral signaling in animals, despite Ral being an oncogenic effector of Ras and altered in RASopathies, a spectrum of related developmental disorders. In conclusion, our study will help better understand Ral exocyst function as well as genetically uncoupling the poorly understood Ras>RalGEF>Ral signal from exocyst functions.

464V ***ztf-16* is a novel heterochronic modulator that opposes adult cell fate in dauer and non-dauer life histories in *Caenorhabditis elegans*** Anuja Dahal[1], Mark A Hansen[1], Taylor A. Bernstein[1], Chani Kohtz[1], Safiyah Ali[1], Aric L Daul[2], Eric Montoye[1], Ganesh P Panzade[3], Amelia F Alessi[4], Stephane Flibotte[5], Marcus L Vargas[2], Jacob Bourgeois[1], Campbell Brown[1], John K Kim[4], Ann E Rougvie[2], Anna Zinovyeva[3], Xantha Karp[11]Central Michigan University, [2]University of Minnesota, [3]Kansas State University, [4]Johns Hopkins University, [5]University of British Columbia

To increase the chance of survival in adverse environments, *C. elegans* transitions into dauer diapause, a stress-resistant, developmentally arrested stage adopted after the second larval molt. Upon return to favorable conditions, dauer larvae exit the dauer stage to resume development into healthy adulthood. However, the mechanisms by which developmental pathways are modulated to accommodate dauer diapause are poorly understood. To study the effect of dauer diapause on development, we focus on the epidermal seam cells. Seam cells are multipotent during larval development but differentiate in adults, a process that is regulated by the heterochronic genes. Interestingly, many heterochronic genes are required only during non-dauer development and are dispensable after dauer, suggesting that a separate developmental pathway controls seam cell development after dauer. To elucidate such a pathway, we conducted a genetic screen for mutants displaying precocious expression of the adult-specific *col-19p::gfp* marker in post-dauer larvae. We found that *ztf-16*, encoding a C2H2 zinc finger transcription factor, is required to prevent precocious *col-19p::gfp* expression beginning in the L1 stage in both dauer and non-dauer life histories. Comparative mRNA-seq analysis also identified 306 differentially expressed genes between wild-type and *ztf-16(-)* larvae. In non-dauer development, the LIN-29 transcription factor directly activates *col-19* and is in turn regulated indirectly by the *let-7* microRNA. Expression of *ztf-16::gfp* was strongly upregulated in *let-7(-)* mutant larvae, indicating that *ztf-16* acts downstream of *let-7.* Genetic epistasis experiments further support this interpretation. However, mutation of the *let-7* site in the *ztf-16* 3'UTR did not affect *ztf-16::gfp* expression, suggesting an indirect regulatory interaction between *let-7* and *ztf-16.* Furthermore, while LIN-29 directly activates *col-19* transcription, *lin-29* was not required for precocious *col-19p::gfp* expression in *ztf-16(-)* larvae, suggesting that additional players are involved in regulating *col-19p::gfp* expression. Taken together, our work describes a novel heterochronic regulator that functions in the dauer life history.

465V **Search for "parasitism genes": comparative transcriptomics of different developmental stages of the nematode *Alloionema appendiculatum*** Violetta Mazakina[1], Boris D Efeykin[1], Anastasia Teterina[21]Center of parasitology, Severtsov Institute of Ecology and Evolution, [2]Institute of Ecology and Evolution, University of Oregon

The phenomenon of parasitism is widespread in nature and prevalent in most taxa. Because the shift to parasitism has occurred repeatedly in the evolution of nematodes, representatives of this taxon engage in a variety of associations, from phoresis to obligate parasitism. Some nematodes have both free-living and parasitic stages in their life cycles, as well as a transitional stage known as the Dauer larva that has pre-adaptations to parasitism and is required for penetration into the host organism. The complex life cycle of nematodes makes them an excellent model to study parasitism and to gain valuable insights into the mechanisms and strategies that parasites use to infect and survive within their hosts.

Studying changes in gene expression during a parasite's life stages provides useful insights into the metabolic pathways that underpin the parasite's functions, supporting the practical goal of identifying potential anthelmintic targets. Several protein families have been recognized as being linked to the transition to parasitism, including those that facilitate immunomodulation (SCP/TAPS, proteinase inhibitors), hinder intestinal contraction mechanisms (acetylcholinesterase), and enable host tissue penetration (propyl oligopeptidase).

In this study, we examined *Alloionema appendiculatum*, the nematode-parasite of terrestrial gastropod mollusks of the genus *Ario*, in the aforementioned developmental phases. We performed transcriptome assembly, followed by study of gene expression of four developmental stages of *A. appendiculatum*, free-living, parasitic, post-parasitic, and invasive. We conducted differential gene expression analysis, enrichment analysis, and functional annotation to uncover pathways linked with parasitism. To contextualize our findings, we compared our results with pathways associated with parasitic developmental stages in model organisms such as the human parasite *Strongyloides stercoralis*, the rat parasite *Strongyloides ratti*, as well as with the free-living nematode *Caenorhabditis elegans*. The present findings help to improve our understanding of the physiological, metabolic, and genetic processes underlying parasitism in nematodes.

466V **Mechanism of Sexual Maturation of the Nervous System in the *C. elegans* Male** Jiarui Zhang[1], Carlos A Diaz-Balzac[2], Maria I Lazaro-Pena[3], Douglas S Portman[31]Biology, University of Rochester, [2]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine University of Rochester Medical Center, [3]Biomedical Genetics, University of Rochester Medical Center

As animals transition from immature juveniles to sexually mature adults, changes in morphology and behavior are precisely timed. While gonadal maturation is the hallmark of sexual maturation, the nervous system undergoes drastic changes as well. In mammals, control of the onset of puberty is not well understood, but involves the RNA-binding protein LIN28B as well as the microRNA LET7, both of which were originally identified in studies of developmental timing in *C. elegans*. We are using temporally regulated neuronal gene expression in *C. elegans* males to investigate the timing of nervous system maturation during the larval-to-adult transition. Previously results have shown that a conserved developmental timing pathway, the heterochronic *lin-28 – let-7* axis, regulates nervous system maturation timing in males. Furthermore, two *lep* genes discovered from forward genetic screens were shown to work upstream of *lin-28* and *let-7 in this process*. *lep-2* is an RNA-binding protein Makorin,

and *lep-5* is a lncRNA. In humans, the Makorin MKRN3 is also implicated in the timing of sexual maturity. Loss of either *lep-2* or *lep-5* delays nervous system maturation by delaying the degradation of LIN-28. These results suggest a working model in which lncRNA *lep-5* acts as a scaffold to bring LEP-2 and LIN-28 into proximity, allowing efficient ubiquitination of LIN-28, which would then be subject to rapid proteasomal degradation. To investigate this working model, we are studying the structure-function relationships of both *lep-2* and *lep-5*. Using Cripsr/Cas9-based genome editing, we will investigate the requirements for specific regions of lncRNA *lep-5*, including candidate LIN-28 binding sites. Moreover, compromising the Zn-binding domains of LEP-2 will provide insight into whether LEP-2 function requires both RNA-binding and E3 ubiquitin ligase activities. Together, these studies will help better understand how *lep-2, lep-5,* and *lin-28* together act as a developmental timer in the nervous system and initiate the juvenile-to-adult transition.

467V **_C. elegans_ and _C. briggsae_ show a species-specific difference in dependence on EGF signaling in both the VPC and P12 cell fate decision** Ashley Castelloe[1], Natalia Kravtsova[2], Helen Chamberlin[2], Adriana Dawes[21]Molecular Genetics, Ohio State University, [2]Ohio State University

Morphological structures of multicellular organisms must be formed appropriately for an organism to survive and reproduce. The formation of these structures relies on appropriate cell fate decisions being made at the right times and by the right cells. Cell fate decisions are dependent upon signaling networks; these highly coordinated and tightly regulated networks of proteins coordinate internal and external feedback to induce the appropriate cell fate that will give rise to a specific morphological structure. One such signaling pathway is the highly conserved EGF pathway. In *Caenorhabditis* worms, this pathway induces the cell fates of the vulval precursor cells (VPCs) and P12 hindgut cell. Reduction of EGF pathway activity via the U0126 MEK inhibitor or mutation of the *sur-2* Mediator complex subunit causes species-specific cell fate phenotypes in *C. elegans* and *C. briggsae* VPCs. It is unknown if reduction of EGF signaling in the P12 causes similar species-specific phenotypes in *C. elegans* and *C. briggsae*. **We hypothesize that EGF pathway activity reduction will result in loss of cell fate in both the *C. elegans* and *C. briggsae* P12, with a stronger response in *C. elegans*.** This will mirror what we see in the VPCs. I tested if targeting different portions of the EGF pathway result in similar species-specific effects in the VPCs and P12. I found that both the VPCs and P12 cell fates show similar defects in response to both U0126 and *sur-2* mutation. In both organs, C. elegans N2 shows more defects than C. briggsae AF16 does. We will test if species-specific cell fate phenotypes of the VPCs and P12 seen in N2 and AF16 are reflected in other *C. elegans* and *C. briggsae* strains by treating with U0126 and tracking the cell fates of VPCs and the P12. Understanding how EGF signaling activity is coordinated across species and organs helps us to understand how cell fate decisions are made and promote morphological structure formation.

468V **Unraveling an ancient mystery: A cooperative catalytic mechanism used by GLH-1 explains the persistent localization of Vasa-family helicases to germ granules** James Bosco[1], Ekaterina Voronina[21]Univserity of Montana, [2]Molecular Biology, University of Montana

ATP-dependent DEAD box RNA helicases are a family of enzymes required for development, reproduction, and are heavily implicated in pathophysiology when dysregulated. The Vasa DEAD box helicase found in Drosophila melanogaster was discovered three decades ago and was the first germline DEAD box RNA helicase to be studied extensively (Sengoku et al., 2006). Mice, humans, and worms all have VASA homologs, with C. elegans possessing 4 total homologs named GLH-1,2,3,4 respectively. In all animals, VASA-family RNA helicases localize to non-membrane-bound cytoplasmic organelles termed germ granules. Previous publications have demonstrated GLH proteins contribute to the integrity of germ granules in the C. elegans germline (Kuznicki et al., 2000; Spike et al., 2008; Marnik et al., 2019). Simultaneous knockout of glh-1 and glh-4 results in near 100% maternal-effect sterility (Spike et al., 2008). We have observed homo- and heterologous interactions among the members of the GLH protein family. By isolating each GLH domain and testing binding interactions for both GLH-1 and GLH-4, we discovered that GLH protein-protein interactions are mediated through the catalytic C-terminal core. We hypothesized that normal function and catalytic activity for GLHs requires dimerization or oligomerization, which has rarely been observed for DEAD box helicases beyond prokaryotic organisms (Huen et al., 2017). To test this hypothesis, we purified and measured the catalytic activity of GLH-1's C-terminal core in vitro with an enzymatic activity assay designed to detect unwinding of small duplex RNAs. From these experiments, we report the first ever cooperative enzymology data for the Vasa helicase family. We propose that these data might explain germ granule disruption observed by Marnik et al., 2019 and Dai et al., 2022 upon expression of a catalytically inactive DQAD mutant in vivo. Furthermore, we hypothesize that the cooperative catalytic mechanism of VASA-family RNA helicases explains the requirement for their localization to germ granules observed across animal species.

469V **IP-MS defines extensive regulators of EGO-1 showing potent *rde* phenotype and 22G siRNA biogenesis identification factor** Farees ud din Mufti[1], Shouhong Guang[21]University of Science & Technology of China, [2]Department of Life Sciences, University of Science & Technology of China

Small RNAs play vital roles in development and gene regulation of eukaryotes. However, the RNAi mechanism is still elusive.

It is also unclear how small RNAs recruit RNA dependent RNA polymerases (RdRPs) to amplify the silencing process in RNAi pathway. We have conducted an immunoprecipitation followed by mass spectrometry (IP-MS) proteomics experiment of EGO-1 and identified several potential interactors. Among them, we focused on C14b1.12, C27b7.5 and other top hit genes. We used fluorescence localization analysis, gene knockdown and knockout to assay their biological roles. We performed deep sequencing of small RNAs in mutants and found that C14b1.12 is a new RNA defective gene (RDE) which may disrupt the P-granule assembly of *ego-1* and named it as ***e**go-1* **d**isruptor **f**actor-1 (*edf-1*). *edf-1* exhibited High Incidence of Male (HIM) phenotype. Deep sequencing of small RNAs in C27b7.5 mutants indicated that C27b7.5 may recruit RdRP complex to mRNA templates and affect 22G small RNA biogenesis.

Key Words: RdRP, functional Proteomics, deep sequencing of small RNAs, CRISPR

470V **Acetylcholine receptors regulate a reversal response in the male gonadal migratory leader cell** Elizabeth Strang[1], Kevin Park[1], Trisha Gongalore[1], Mihoko Kato[21]Pomona College, [2]Biology, Pomona College

The migration of the linker cell (LC), the male gonad leader cell, determines the shape of the developing gonad and guides it to the cloaca. We previously reported that the LC expresses and uses the acetylcholine (ACh) muscarinic receptor *gar-3* during its migration. In particular, when *gar-3* was overactivated using aldicarb, an ACh esterase inhibitor, the L4 stage LC reversed its orientation from the normal posterior-facing to an anterior-facing cell, based on its morphology and intracellular organization. Here we addressed whether nicotinic receptors are also necessary for this reversal response, since aldicarb can activate both receptors types, and found that they are. When aldicarb is replaced by either muscarinic or nicotinic agonists, the reversal does not occur, but the combination of both reverses the linker cell orientation. The transcriptome of the LC contains seven nicotinic receptors, among which *lgc-9*, *acr-15*, and *acr-16* are expressed much higher relative to the others. We confirmed the expression of *lgc-9::gfp*, and previously *acr-16::gfp*, in the LC. We tested these three receptor knockouts individually and as a triple mutant and found that the LC still reverses in the absence of these receptors, suggesting that other nicotinic receptors function redundantly. All nicotinic receptors show almost exclusively L4 stage expression in the LC, and this may explain why only L4 stage LCs have a reversal response to aldicarb.

We also investigated which subset of cholinergic neurons might be the source of ACh for the LC, and whether proximity to the path of LC migration was a factor. The ventral nerve cord (VNC) runs parallel and proximal to the L4 stage LC path providing a potential local source of ACh. We previously showed that cholinergic neuronal function was important using the ACh transporter mutant, *unc-17*, which does not reverse its LC orientation in response to aldicarb. Here we tested transcription factor mutants *unc-86* and *unc-3*, which are required to specify cholinergic fate to a subset of neurons in the head and ventral nerve cord, respectively, and found that neither were required for the LC reversal response. This was particularly surprising for *unc-3*, which retains only a small fraction of cholinergic neurons in the VNC, eliminating most cholinergic neurons proximal to the LC path. We suggest that the LC uses ACh released into the pseudocoelom rather than a local, diffusible source.

471V **Comparison between phase-field model and coarse-grained model for characterizing cell-resolved morphological and mechanical properties in a multicellular system** Guoye Guan[1], Xiangyu Kuang[1], Chao Tang[1,2,3], Lei Zhang[1,4,51]Center for Quantitative Biology, Peking University, [2]Peking-Tsinghua Center for Life Sciences, Peking University, [3]School of Physics, Peking University, [4]Beijing International Center for Mathematical Research, Peking University, [5]Center for Machine Learning Research, Peking University

Embryonic development is a precise and complex process involving the cell morphology and mechanics interacting in space and time. The difficulty in quantitatively acquiring cellular morphological and mechanical information *in vivo* makes mathematical modeling a challenging problem and impedes model validation. Recently, the three-dimensional time-lapse live imaging and delineated developmental programs in the roundworm *Caenorhabditis elegans* provide an excellent platform for establishing quantitative models. In this paper, we study two popular computational models for multicellular systems, *i.e.*, the phase-field model and the coarse-grained model, and compare their performance in characterizing the cell morphologies, cell adhesion, and cell stiffness in a real *C. elegans* embryo. We show that both models can capture cell-cell contact areas and heterogeneous cell adhesion, but only the phase-field model succeeds in inferring the heterogeneous cell stiffness by fitting cell shapes or cell-cell interface curvatures. Moreover, we demonstrate that the phase-field model converges to the coarse-grained model when increasing cell surface tension to dominance, obtaining a distance-dependent isotropic intercellular force.

Reference: Guoye Guan[†], Xiangyu Kuang[†], Chao Tang[*], Lei Zhang[*]. Comparison between phase-field model and coarse-grained model for characterizing cell-resolved morphological and mechanical properties in a multicellular system. *Commun. Nonlinear Sci. Numer. Simul.*, 117: 106966 (2022)

472V **Expression and function of recently duplicated genes at specific developmental stages in *C. elegans*** Fuqiang Ma, Chun Yin Lau, Chaogu ZhengSchool of Biological Sciences, the University of Hong Kong

Gene duplication produces the materials for the origin of evolutionary novelties in many species. However, the mechanisms that mediate the retention of the duplicate genes are not clear. In particular, what biological processes involve the newly generated duplicate genes? We addressed this question in *C. elegans* by analyzing the expression pattern and potential functions of the recently duplicated genes. We first used the genomic data of eleven *Caenorhabditis* species to identify genes duplicated at different time points in evolution and generated lists of ancient, old, and young duplicate genes, as well as single-copy genes. Using bulk-RNA sequencing data and hierarchical clustering, we found that young duplicated genes are preferentially expressed in specific developmental stages, including early embryos, late embryos, and late larval stages. In contrast, old genes and single-copy genes showed much less expression dynamics across development. Single-cell transcriptomic analysis further identified enriched expression of the young genes in specific lineages and cell types. For example, in the early embryos, young duplicate genes appeared to show highly enriched expression in the AB lineage, while in late embryos and larval stages, young genes tend to show enriched expression in muscle cells, hypodermis, and seam cells, as well as sperms. Among the differentiated neurons at the L4 stage, young genes tend to be enriched in sensory neurons. Moreover, based on both RNAi and allele phenotypes, we found that the young duplicate genes tend to be less essential than old genes and single-copy genes; and the few essential young genes showed enriched expression in early embryos, which is consistent with their potentially essential functions in embryonic development. Collectively, our work identified the expression of recently duplicated genes at specific development stages and in specific tissues. These results lead to the hypothesis that young genes may contribute to the morphological divergence in early embryonic development among the nematode species, facilitate the differentiation of certain tissues in late embryos, and play a role in chemosensory perception to enhance adaptability to external environment.

473V **VAB-8 and EFN-4/Ephrin act cell-autonomously downstream of MAB-5/Hox to drive QL posterior migration** Vedant Jain, Vitoria Paolillo, Matthew Ochs 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 initiation of the 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 for decades. 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-6* 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. Initial imaging suggests that *vab-8* and *efn-4* mutants fail to extend large posterior protrusions that are characteristic of QL.a and QL.ap posterior migration in wild-type. 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.

474V **Automated analysis of cellular morphology with resolved cell identity throughout *C. elegans* embryogenesis** Guoye Guan[1], Yiming Ma[2], Jianfeng Cao[3,4], Zelin Li[3,4], Vincy Wing Sze Ho[2], Lu-Yan Chan[2], Ming-Kin Wong[2], Hong Yan[3,4], Chao Tang[1,5,6], Zhongying Zhao[2,71]Center for Quantitative Biology, Peking University, [2]Department of Biology, Hong Kong Baptist University, [3]Department of Electrical Engineering, City University of Hong Kong, [4]Center for Intelligent Multidimensional Data Analysis, City University of Hong Kong, [5]Peking-Tsinghua Center for Life Sciences, Peking University, [6]School of Physics, Peking University, [7]State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University

Change in cell shape over development is of central importance for morphogenesis and organogenesis. It is a challenging task to systematically characterize cellular morphology over development. Using 4D images of ubiquitously labeled cell membranes and nuclei, we previously established a platform that allows systematic measurement of cellular morphology, including shape, volume, surface, and contact area, up to the 350-cell stage of *C. elegans* embryogenesis. However, the image segmentation algorithm demonstrates a sharp increase in error rate after the 350-cell stage, making it impractical to characterize cellular morphology further into embryogenesis. Here we present a new segmentation method by taking advantage of the nucleus position along with a new transgenic strain with better optimized expression, which shows much improved accuracy in segmenting cell

membranes up to the 550-cell stage when most embryonic cells complete their last round of division with defined fate. A total of 1292 cells (96.78% out of all cells produced in *C. elegans* embryogenesis) have been successfully segmented with resolved cell identity, including 105 cells destined to be apoptotic ones, which allows systematic and quantitative analysis of cell shape, volume, surface, and contact area. To demonstrate the application of the newly developed platform, we quantified cell volume asymmetry between an apoptotic cell and its sibling. We found 85% of 94 cell pairs demonstrate a sharp volume asymmetry immediately after their birth. We further demonstrate that the Notch signaling pathway regulates the asymmetry of fate along with volume. To facilitate access to our data, we built a standalone software, *CVE*, for customized visualization of cell shape, volume, surface, and contact area data. In summary, we establish a new platform that allows qualitative and quantitative analysis of cell shape, volume, surface, and contact area up to the last round of embryonic division in *C. elegans*, which is expected to facilitate the study of cellular interactions with single-cell resolution at a 1.5-minute interval.

475V **Sex-determination in the male/female species *C. nigoni*** Jonathan P Harbin, Ronald EllisRowan SOM

The emergence of self-fertility in *Caenorhabditis* is ideal for investigating the origin of new traits, since it evolved recently in three separate lineages. In each case, the self-fertile hermaphrodites are *XX* animals that gained the ability to make sperm during larval development, but still produce oocytes during adulthood. Characterizing the sex-determination pathway in the male/female species *C. nigoni*, which represents the ancestral state of the genus, will help us identify the genetic modifications that cause spermatogenesis in *XX* hermaphrodites of its sister species *C. briggsae.* Characterizing these modifications is essential for defining the changes needed to produce self-fertility.

Using a reverse genetic approach, I generated *C. nigoni* mutations in critical sex-determining genes. The first gene I targeted was the putative master regulator of the sex-determination pathway, *Cni-tra-1,* which encodes a Gli transcription factor. *C. nigoni tra-1(v481)* masculinizes the somatic tissues of *XX* animals. Moreover, it appears to arrest gonad development and these *Cni-tra-1 XX* animals cannot sire progeny. Although mutations in *C. elegans* or *C. briggsae tra-1* also cause *XX* animals to develop male bodies, they become fertile males, producing gonads with sperm and oocytes. Surprisingly, older *Cel-tra-1* or *Cbr-tra-1 XO* males often make sperm and oocytes too. This *XO* switch might be a consequence of self-fertility, as *Cni-tra-1 XO* animals have not been seen producing oocytes.

When *C. elegans* or *C. briggsae XX* animals are homozygous null for *tra-2,* they develop imperfect male bodies, but produce only sperm. *Cni-tra-2* also promotes female fates, since *tra-2(v498) XO* animals develop normally but the *XX* animals become imperfect males that produce only sperm. Surprisingly, heterozygosity for *tra-2* induces spermatogenesis in *C. elegans* or *C. briggsae* female mutants, but it does not affect *C. nigoni* females. Thus, low levels of TRA-2 in germ cells might promote self fertility.

My third target was *Cni-fem-3,* which encodes a novel protein that promotes male development in *C. elegans*. The ability of FEM proteins to promote spermatogenesis at multiple points in the sex-determination pathway might be species-specific, as *C. briggsae* fem genes are not required for spermatogenesis, whereas they are in *C. elegans*. The *Cni-fem-3(v496) XO* animals have a feminized somatic body and germline, producing only oocytes. This phenotype differs from the sister species *C. briggsae*, where the *fem-3(nm63) XO* animals become hermaphrodites.

All of these *C. nigoni* mutants were generated in the inbred strain JU1422, which was used for the first *C. nigoni* genome sequence. I›m currently generating more alleles in the distantly related strain CP168, whose draft genome was just shared with us by E. Haag and E. Schwartz. Final characterization for each gene will use JU1422/CP168 hybrids to reduce any background effects.

476V **Maintenance of neuronal identity by Hox proteins through a homeostatic mechanism** Weidong Feng, Honorine Destain, Catarina Catela, Yihan Chen, Paschalis Kratsios Neurobiology, University of Chicago

Hox transcription factors play fundamental roles during early patterning, but they are also expressed continuously, from embryonic stages through adulthood, in the nervous system. However, the functional significance of their sustained expression remains unclear. In *C. elegans* motor neurons (MNs), we found that LIN-39 (Scr/Dfd/Hox4-5) is continuously required during post-embryonic life to maintain key features of neuronal terminal identity, such as the expression of neurotransmitter biosynthesis proteins, ion channels and neuropeptides. ChIP-Sequencing combined with genetic analysis revealed that LIN-39 activates directly terminal identity genes in MNs. We further show that LIN-39, MAB-5 (Antp/Hox6-8) and the transcription factor UNC-3 (Collier/Ebf) operate in a positive feedforward loop to ensure continuous and robust expression of MN identity genes. Importantly, we identified a two-component design principle for homeostatic control of Hox gene expression in adult MNs: Hox transcriptional autoregulation is counterbalanced by negative UNC-3 feedback. Lastly, our findings are conserved in the mouse spinal cord. We found that *Hoxc8* is not only required to establish, but also maintain at later stages the expression of terminal identity genes in spinal MNs. Altogether, our findings in *C. elegans* and mice uncover a noncanonical and evolutionarily conserved role for Hox proteins during post-embryonic life, critically broadening their functional repertoire from early patterning to the establishment and maintenance of neuronal terminal identity.

477V **Revisiting hox gene evolution, and Hox cluster linkage across Nematoda** JOSEPH KIRANGWA[1], Dominick Laetsch[2], Earna King[3], Mark Blaxter[3], Oleksandr Holovachov[4], Philipp H. Schiffer[51]Biomedical sciences, University of Cologne, [2]University of Edinburgh, [3]Wellcome Sanger Institute, [4]Swedish Museum of Natural History, [5]University of Cologne

Hox genes occupy a prime position towards our understanding of metazoan body plan formation, patterning and evolution. Traditionally, nematodes are often considered different because C. elegans is different: Here we have re-visited the Hox gene complements of Nematoda not only to understand the evolution of this key set of body pattern genes using newly available high-quality genomes but also to classify the loci into their ortholog groups as well as look for linkage between loci.

Previously, genomes from Enoplea as well as early branching Chromadorea were not investigated due to a lack of genomic resources. We have used newly sequenced high-quality draft genomes together with those already available in the public databases to perform sequence similarity search methods.

Our analysis of Hox gene complements spanning the Nematoda revealed Hox gene gains and losses, different orientations within the clusters and that the arrangements on the clusters can be interrupted by unrelated genes. Our findings might point towards diversified roles of Hox genes during development extending beyond the boundaries of providing spatial information. The presence of interrupting unrelated genes with no obvious orthologs is suggestive of ongoing Hox cluster expansion in the analyzed nematode genomes.

Altogether, Nematoda Hox genes are orthologous to 6 Drosophila Hox genes; labial, Hox3, deformed, fushi tarazu, antennapedia and Abd-B. Furthermore, we found that C. elegans misses two Hox genes (Hox3 and Hox6-8/Antp) which are present in ancestral nematode lineages. While the core C. elegans Hox cluster is loosely connected, we found condensed Hox gene clusters in Nematode genomes belonging to Clades currently considered to be ancestral to C. elegans. Furthermore, we found that the two Hox genes ceh-13 and lin-39 deviate with respect to the arrangement in the Hox cluster in that lin-39 is more distal than the labial-like gene ceh-13 in C. elegans, Bursaphelenchus xylophilus, Onchocerca volvulus and Brugia mulayi. However, in species belonging to early branching groups, such as Plectus sambesii, Sabatiera pulchra and Trichuris suis, the anterior ceh-13 lays upstream of lin-39. This finding illustrates the evolutionary dynamics of the Hox cluster in Nematoda. Further research regarding nematode Hox gene complements that we have identified herein will seek to understand the link between genomic and expression patterns of Hox genes in various non-model nematode species.

478V **Epidermal ribosome synthesis inhibition induces a nutritional uncoupled organism-wide quiescence in *C. elegans*** Qiuxia Zhao[1], Rekha Rangan[2], Shinuo Weng[3], Cem Ozdemir[2], Elif Sarinay Cenik[11]Molecular Biosciences, University of Texas, Austin, [2]Molecular Biosciences, UT Austin, [3]Molecular Biosciences, Ut Austin

Inter-organ communication plays a crucial role in the growth, development, and maintenance of homeostasis in multicellular organisms. However, the cellular non-autonomous cues that regulate tissue-specific growth are poorly understood due to limitations in cell ablation techniques. In this study, we present a method for investigating organism-wide growth coordination independent of nutrition by selectively controlling ribosome biogenesis in a tissue-specific and reversible manner in *Caenorhabditis elegans*. Our results show that suppression of ribosome synthesis, either through depletion of an RNA polymerase I subunit or two critical chaperone proteins involved in ribosome assembly, leads to an organism-wide growth quiescence response. This response is not influenced by the insulin signaling pathways or rescued by a mutation that suppresses the starvation-induced quiescence response. Upon investigating the tissues involved in this process, we discovered that inhibiting hypodermal ribosome synthesis alone was sufficient to trigger the organism-wide growth quiescence response and result in changes in gene expression across various cell types, including touch receptor neurons. This suggests inter-organ communication upon ribosome inhibition in the hypodermis. Additionally, we found that dense core vesicle secretion from the hypodermis tissue, specifically through the *unc-31* gene, plays a significant role in mediating the observed quiescence phenotype. These results indicate the presence of a multicellular growth coordination mechanism that is independent of nutrition and initiated from the hypodermis tissue.

479V **Organelle specific V-ATPase pumps with distinct functions in unicellular tubulogenesis** Liakot A Khan, Gholamali Jafari, Edward A Membreno, Verena A GobelPediatrics, Massachusetts General Hospital and Harvard Medical School

Eukaryotic cell compartmentalization allows distinct functions to be segregated in different membrane-bound organelles. V-ATPases are membrane-embedded proton pumps that acidify such membrane-bound compartments and have various cellular functions. A full V-ATPase pump consists of 13 or 14 subunits, several of which have different isoforms, and some of these isoforms are encoded by different genes. Different subunit isoforms determine the tissue specificity of V-ATPase pumps but can also determine subcellular organelle specificity, a function chiefly attributed to subunit ‘a’ isoforms. Little is known about the number, subunit composition and functional coordination of organelle-specific pumps within a single cell in multi-cellular organisms.

We have identified multiple V-ATPase subunit genes required for the development of the unicellular *C. elegans* excretory canal. The expression analysis of 8 of these genes unexpectedly revealed their distribution to several distinct membrane compartments within this single cell. Compartment-specific localization was noted for subunits of the V0 and V1 V-ATPase domains, subunits with only one isoform, and subunits with several isoforms, including non 'a' subunit isoforms. For instance, among subunit 'c' isoforms, VHA-1 chiefly localizes to canal-specific canalicular endo-membranes, VHA-2 to the basal plasma membrane, and VHA-3 to vesicular endo-membranes.

The analysis of several of these V-ATPase subunit genes revealed different subunit-specific functions. Depleting canalicular VHA-1 and VHA-5 results in the absence of canal growth and lumen formation, whereas depleting VHA-8 and VHA-20, both localized at the apical membrane, causes lumen enlargement and cyst formation. Canalicular, but not apical, V-ATPase subunit isoforms are required to recruit AQP-8/aquaporin, SULP-4, and ERM-1 to the apical membrane (lumen), but not SULP-8 to the basal membrane. Endo- and plasma-membrane associated subunit isoforms differentially affect the dynamics of early and late endosomes, secretory vesicles, and recycling endosomes. Collectively, our findings suggest that endo-membrane-based pumps direct apical membrane components and flux to expand the lumenal membrane, while apical-membrane-based pumps function in endocytic-recycling to limit apical membrane expansion, thereby equilibrating the lumen diameter. Our findings also suggest that the range of V-ATPase pumps with different subunit composition, subcellular localization and function within a single cell is larger and more complex than currently appreciated.

480V **The role of 3'UTR regulation of *ifet-1* in early *C. elegans* development.** Lu Lu, Claire Nuessmeier, Henry Neri, Rita Okeke, Carmela Rios, Allison Abbott Biological Sciences, Marquette Univ

Translational control of gene expression in germ cells is essential for normal gametogenesis and embryogenesis. Maternal mRNAs in *C. elegans* are repressed in P granules in germ cells and then are selectively activated during oogenesis and early embryogenesis to allow for the proper specification of early blastomeres. IFET-1 is an eIF4E binding protein that localizes to germ granules and functions as a translational repressor of a set of target maternal mRNAs, including *mom-2* and *zif-1.* Interestingly, IFET-1 in the early embryo is rapidly degraded, becoming restricted to the germ cell lineage after the four cell stage. Although the activity of IFET-1 plays an important role in the regulation of maternal mRNA targets, and therefore influences germline development and early cell fate specification, the regulatory mechanism of *ifet-1*expression is largely unknown. We hypothesized that 3'UTR-mediated regulation could potentially control *ifet-1* levels in the early embryo. Using CRISPR genome editing tools, we constructed two *ifet-1* 3'UTR mutant alleles, *xw62* and *xw63*, to study 3'UTR regulation of *ifet-1.* One allele, *xw62*, was created to remove binding sites for a subset of male gonad-enriched microRNAs. The second allele, *xw63*, was created to have a large region of the *ifet-1* 3' UTR deleted. In *ifet-1(xw62)* mutants, we observed a lower average brood size and a reduced number of sperm in hermaphrodites. Whereas in *ifet-1(xw63)* mutants, we observed stronger brood size defects, embryonic lethality, and adult lethality largely resulting from egg laying defects. mRNA levels are elevated in *ifet-1(xw62)* and *ifet-1(xw63)* mutants in L4 and adult stage worms. Using transgene reporters, two maternal mRNAs, *mom-2* and *zif-1*, were shown to have a delayed activation in *ifet-1(xw63)* compared to control. Together, these data suggest that 3'UTR of *ifet-1* is necessary to promote the downregulation of *ifet-1* and proper control of maternal mRNAs in the early embryo. Future work will focus on identifying specific regions of the 3' UTR that are necessary and sufficient for this regulation.

481A **Harnessing natural genetic variation to identify structure-specific molecular mechanisms of per- and polyfluoroalkyl substances (PFAS) toxicity** Tess Leuthner[1], Ryan Baugh[21]Duke University, [2]Biology, Duke University

An estimated 200 million US residents are drinking water contaminated with per- and polyfluoroalkyl substances (PFAS) and 99% of all human blood serum samples tested in the US contain PFAS. Epidemiological evidence suggests that exposures to these "forever chemicals" are associated with major diseases, including cancer. However, the mechanisms of toxicity of practically all the >12,000 emerging, structurally complex PFAS remain entirely unknown. Therefore, we leveraged natural genetic variation of wild *C. elegans* to identify structure-specific molecular mechanisms of PFAS toxicity. We are investigating toxicity of PFAS chemicals that vary in three structural attributes (chain length, functional group, and chain composition) and the contribution of natural genetic variation on response to PFAS exposures. I conducted a toxicity assay in N2 to determine the effective concentration in which a 48 hr exposure resulted in a 50% reduction in growth (EC50). There was significant variation in EC50 values among PFAS chemicals. This experiment was repeated using 12 genotypically different strains from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR). We observed variation in toxicity (EC50 values) among strains within PFAS chemicals, suggesting that underlying genetic variation causes variation in response to exposures. Next, I will use a pooled-population (192 strains) and bulk-sequencing approach to identify the sensitivity or resistance of each strain to each chemical to identify quantitative trait loci (QTL). I will then use various genetic and genome-editing approaches to explicitly identify the role of one or more gene variants that contribute to structure-specific toxicity of PFAS chemicals. Overall, this approach demonstrates the power of using genetics to analyze mechanisms of toxicity.

482A **Species-specific loss of a gene involved in protein glycosylation leads to complete embryonic lethality in the hybrids**

between *Caenorhabditis briggsae* and *C. nigoni* Dongying Xie, Yiming Ma, Pohao Ye, Gefei Huang, Yiqing Liu, Tongwen Wen, Zhongying Zhao Hong Kong Baptist University

Post zygotic hybrid incompatibility (HI) including hybrid sterility and lethality precludes gene flow between species or populations, which eventually leads to speciation. Despite intensive studies of speciation genetics during the past decades, molecular identity has been established only for a very few HI loci across all the taxa, preventing a thorough understanding of the mechanism underlying speciation. No single interspecies HI gene has been molecularly defined although a handful of intraspecies HI genes were cloned in nematodes. We therefore aimed to molecularly clone HI genes between nematode species. Using *C. briggsae* and its sibling species *C. nigoni* as a model, we previously identified genome-wide HI loci between the two species by repeatedly backcrossing different GFP-linked *C. briggsae* genomic fragments into *C. nigoni*. This produced 112 *C. nigoni* introgression strains, each of which carried a GFP-linked *C. briggsae* genomic fragment in an otherwise *C. nigoni* background. A complete lethality was observed in the GFP-expressing hybrid embryos between *C. briggsae* and one of the *C. nigoni* introgression strains bearing the right arm of *C. briggsae* chromosome IV, whereas the hybrid embryos between wild isolates of the two species are viable. We previously mapped the hybrid lethal locus to an interval of approximate 6 Mb through backcrossing. Here we successfully narrowed down the interval to approximate 40kb on the *C. nigoni* chromosome IV by CRISPR/cas9 mediated targeted recombination. The molecular identity of the hybrid lethal locus was defined by a combination of systematic knockouts and complementation tests, which carries an single gene *Cni-helh-1* (Homozygosity leads to complete Embryonic Lethality in Hybrid). Hybrid embryos between *C. briggsae* and *C. nigoni* carrying a *Cni-helh-1* null allele were completely inviable, indicating that loss of *C. nigoni*-specific *helh-1* leads to the complete lethality. The fully penetrant embryonic lethality in the hybrids could be fully rescued by heterogenous expression of *Cni-helh-1* through knock-in assay. *helh-1* encodes a conserved enzyme essential for the synthesis of uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc), a precursor for glycosylation and chitin synthesis. We further showed that the hybrid lethality appeared to be caused by a negative epistatic interaction between *Cbr-helh-1* and another unknown *C. nigoni* element/factor that was also linked to the chromosome IV, which fits well with Dobzhansky Muller (DM) model. Our results demonstrate the cloning of an interspecies HI gene between *Caenorhabditis* species for the first time, and reveal the importance of glycosylation pathway during speciation.

483A **Cultivating *C. elegans* in its true ecological niche: a peek into nematode-microbe interaction in a laboratory-based "natural habitat"** Rocel Amor Indong[1], Jongmin Park[2], Jin Lee[2], Tae Kwon Lee[2], Jin-Kyung Hong[2], Eun Sun Lyou[21]Division of Biological and Technology, Yonsei University Mirae Campus, [2]Yonsei University Mirae Campus

It has long been established that animals evolve to adapt to their natural environment. Environmental changes - ranging from physicochemical factors to changes in behavior of cohabiting organisms - cause animals to change their behavior and physiology to increase their reproductive fitness and survival. However, while these behavioral changes in animals have been reported to stem from their genes, the interplay between natural ecology and the gene is challenging to ascertain. *Caenorhabditis elegans*plays an important role in studying gene-controlled behavior since it is the primary genetic model organism for the last 50 years. Nonetheless, little is known about the natural ecology of *C. elegans* and how environmental cues affect its behavior. This study aims to establish a novel protocol where *C. elegans* ecology, behavior, and physiology can be observed in a simulated natural habitat in the laboratory. We developed a soil-apple-natural-habitat (soil-fruit habitat) that simulates the true ecological niche for *C. elegans*. Apples were placed on potted-soils and subjected to day/night, temperature, and humidity cycling. After a period of apple-rotting, 30 L1 larvae were added to the soil surface and the growing worm population was observed per layer of the apple and soil. Our own concoction of microbial solution (MS) was made by washing a mixture of rotting apple and soil. Preliminary data showed that the soil-fruit habitat set-up allows normal growth and reproduction of *C. elegans*. MS titration showed that low concentration resulted in limited population growth within 9 days while higher concentration allows for continuous population growth for 15 days. We found that the presence of the apple contributes significant sugar content, causes a two order of magnitude increase in microbial cells, and significantly enriches *C. elegans* population growth. Interestingly, in some of our preliminary experiments we also observed that the presence of *C. elegans* populations can drive phenotypic diversity of microbial communities in both soil and fruit as the ecological experiment progressed over time. We are currently analyzing the effect that *C. elegans* populations have on the microbial profile using NGS. We will also vary environmental conditions such as temperature, humidity and light cycling and assess *C. elegans* growth in the soil-fruit habitat. Overall, we hope this protocol will help in elucidating the relationship between genetics, behavior and physiology, and the natural ecology of the nematode.

484A **Disease-decreasing diversity: more biodiverse *C. elegans* communities contain fewer nematode-infecting pathogens** Robbert van Himbeeck[1], Jessica N Sowa[2], Hala Tamim El Jarkass[3], Wenjia Wu[4], Job Oude Vrielink[1], Joost A G Riksen[1], Aaron Reinke[3], Lisa van Sluijs[11]Laboratory of Nematology, Wageningen University and Research, [2]Department of Biology, West Chester University of Pennsylvania, [3]University of Toronto, [4]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Coastal Zones, South China Botanical Garden, Chinese Academy of Sciences

Natural populations of *Caenorhabditis elegans* contain many co-occurring nematodes and also pathogens that infect them, such as oomycetes, microsporidia and viruses. In macroscopic species, differences in pathogen susceptibility of hosts contribute to the 'dilution effect' where higher host biodiversity contributes to lower pathogen prevalence, but this mechanism is unidentified for microscopic species. Here, we unraveled wild *C. elegans* communities to investigate the effect of nematode biodiversity on the prevalence of nematode-infecting pathogens. Natural nematode communities including *C. elegans* were collected from decaying plant substrates. Next, nematodes in these communities were fully characterized, mostly to species-level resolution, after the nearly complete eukaryotic SSU was PCR-amplified and sequenced by Nanopore sequencing. Using (wild) *C. elegans* as a target species, we could use fluorescent reporter strains to identify bacterial, microsporidian and viral pathogens present, including potentially novel species. Together, our data indicates that communities rich in nematode species contained fewer specialist pathogens than those with low nematode biodiversity. Future work will use collected *C. elegans* and other nematode species for multispecies infection experiments to establish which factors drive the 'dilution effect of disease'.

485A **Genomic signatures of asexual reproduction in *Diploscapter* nematodes** George Chung[1], Lewis Stevens[2], Karin Kiontke[1], Manuela R Kieninger[2], Fabio Piano[1], David H. A. Fitch[1], Mark Blaxter[2], Kristin C Gunsalus[11]Biology, New York University, [2]Tree of Life, Wellcome Sanger Institute

Diploscapter pachys is a parthenogenetic nematode from a long-lived lineage with an abridged meiosis and a highly heterozygous genome. It and several related parthenogenetic nematodes have an unusual karyotype of 2*n* = 2 (Fradin, Kiontke, Zegar et al. 2017 *Current Biology*, Hiraki *& al*. 2017 *BMC Genomics*). To understand the evolutionary trajectory of *D. pachys* from sexual reproduction to parthenogenesis, and to understand the link between its unusual karyotype and the parthenogenetic mode of reproduction, we are undertaking a comparative analysis of genome evolution across the clade of parthenogenetic *Diploscapter/Protorhabditis* species. For each species, we are generating a phased diploid, chromosome-level assembly using long-read sequencing (Oxford Nanopore Technologies or PacBio) complemented with scaffolding using chromatin contact capture (Pore-C or Hi-C). We have uncovered a complex relationship among the parthenogenetic *Diploscapter* species based on their genomic relatedness. Furthermore, we present the evidence for linear *Diploscapter* chromosomes with an unexpected diversity of telomere repeat sequences and with a history of fusion and rearrangements of ancestral nematode chromosomes. Finally, we present an updated view on the evolution of *Diploscapter* and its parthenogenetic mode of reproduction.

486A **Radioactivity of nematode collection site in Chernobyl Exclusion Zone does not predict mutation load or mutagen tolerance** Sophia Tintori, Matt Rockman New York University

Populations that are chronically exposed to above-average levels of radiation may exhibit elevated mutation rates, and they may adapt to such an environment via selection on DNA repair variants. To test these hypotheses we collected nematodes from areas of the Chernobyl Exclusion Zone ranging in radioactivity.

We isolated, cultured, identified, and cryopreserved 298 lines from eight nematode clades, including many *Oschieus*, *Panagrolaimus*, and *Acrobeloides*. We assembled genomes *de novo* for 15 *Oschieus tipulae* strains using long-read data, and saw no evidence for mutation abundance or spectrum scaling with radiation levels at the site of collection, or corresponding to whether worms were collected from Chernboyl on non-Chernobyl regions.

We tested these same 15 strains from Chernobyl and several non-Chernobyl strains for tolerance to several types of DNA damaging agents. We found that the most tolerant and the most sensitive strains were different depending on the mutagen, and that mutagen tolerance did not scale with radiation level at the site of collection.

These results do not support a hypothesis that surviving worms from Chernobyl worms are either more mutagenized or more resistant to mutagens than other worms of the same species. Regardless, the result do points us towards specific strains that are reproducibly and heritably more resistant to different types of mutagens.

487A **Natural variation in *Caenorhabditis elegans* egg-laying behaviour modulates an intergenerational fitness trade-off** Laure Mignerot[1], Clotilde Gimond[2], Lucie Bolelli[3], Charlotte Bouleau[3], Asma Sandjak[2], Thomas Boulin[41]Institut Biologie Valrose, CNRS, UCA, [2]CNRS, [3]CNRS, UCA, [4]Insitut NeuroMyoGène, CNRS

Evolutionary transitions from oviparity to viviparity are frequent across diverse taxa. Many species display intraspecific variation in egg retention, that is, an intermediate type of parity by laying eggs containing embryos at advanced stages of development. How such natural quantitative variation in egg retention arises through differences in genetics, behaviour, and physiology – and how this variation ultimately connects to variation in specific fitness components – is poorly understood. We address this problem by characterizing intraspecific variation in constitutive retention of fertilized eggs of the nematode *Caenorhabditis elegans*. Analysing a panel of ~300 wild strains, we report highly variable intra-uterine retention of fertilized eggs, with a fraction of strains showing either strongly reduced or increased egg retention with partial viviparity. We provide evidence for multiple

evolutionary origins of such phenotypic extremes and identify candidate QTL (Quantitative Trait Loci) by genome-wide association mapping underlying natural variation in egg retention. Characterizing a subset of wild strains, we confirm that natural variation in egg-laying behaviour contributes to observed differences in egg retention. Using multiple neuromodulatory agents and controlled CRISPR-*Cas9*-mediated genetic manipulation of endogenous serotonin levels in 10 wild strains, we show that this behavioural variation arises through an evolutionarily divergent neuromodulatory architecture of the egg-laying circuitry. Intraspecific variation in *C. elegans* neural circuit activity hence connects with variation in reproductive strategies, including transitions from oviparity to partial viviparity. We then aimed to explore why natural variation in *C. elegans* egg retention may be maintained. Examining potential fitness costs and benefits of this natural variation, we show that strong egg retention reduces maternal fertility and survival, mostly due to detrimental larval hatching *in utero*. On the other hand, such genotypes with strong egg retention can benefit from improved offspring protection against environmental insults and by gaining a competitive advantage as offspring exhibit a shortened extra-uterine developmental time to reproductive maturity. Observed natural variation in *C. elegans* egg-laying behaviour may thus reflect modifications of a trade-off between alternative fitness components expressed across generations. Our study uncovers underappreciated levels of natural diversity in the *C. elegans* egg-laying circuit and provides insights into the fitness consequences and potential adaptive significance of this behavioural variation. We propose that intraspecific variation in nematode egg-laying behaviour can be used as a suitable system to pinpoint the molecular causes underlying evolutionary transitions between invertebrate ovi- and viviparity.

488A **Natural microbiome protects against paralysis in an amyloid-beta model of disease** Feng Xue[1], Mireya Vazquez-Prada[1], Nathan Dennis[1], Laura Freeman[1], Andreea Aprodu[2], Filipe Cabreiro[2], Marina Ezcurra[11]University of Kent, [2]University of Cologne

The gut microbiome is known to be important in maintaining host health and modulating the progression of chronic diseases. Studies have associated dysbiosis of the gut microbiome with a range of diseases including neurodegenerative diseases, although the underlying molecular mechanisms are not known. Understanding such host-microbiome interactions can enable the development of microbiome-based therapies to treat these diseases.

In this project, we examine how amyloid-beta toxicity is influenced by the host microbiome. We use a *C. elegans* disease model in which amyloid-beta is expressed in the body wall muscle that leads to progressive aggregate-formation and paralysis. Aggregation of amyloid-beta is a major pathological feature of Alzheimer's diseases, one of the most common neurodegenerative diseases which currently has no cure.

With this model of amyloid-beta, we explored effects of the natural *C. elegans* microbiome on paralysis, concentrating specifically on an experimental microbiome consisting of 11 select bacterial species, cultured from wild isolates of *C. elegans*. This experimental microbiome suppresses the onset of paralysis compared to *E. coli* OP50, indicating that it has a role in modulating proteostasis in the host. The cell-free media from this experimental microbiome also suppresses paralysis and inhibits aggregation of amyloid-beta in vitro, indicating the involvement of extracellular components. Within the experimental microbiome, we identified a single isolate species that demonstrates strong suppression of amyloid-beta proteotoxicity and aggregation. We are using multiple strategies to identify the bacterial compounds and signalling pathways responsible for this protection against amyloid-beta pathology. This includes comparing closely related species to determine genotype-phenotype associations; generating a transposon-insertion library for screening of genetic mutants; and testing predicted candidate genes and pathways.

489A **Divergence and similarities of the embryonic transcriptomes of nematodes** Tarja Tamara Hoffmeyer[1], Viktoria Hellekes[1], Sarah Schiedewitz[2,3], Sebastian H. Schlede[2], Denise Claus[2], Naïra Sarkis[2], Philipp H. Schiffer[11]Developmental Biology, University of Cologne, [2]University of Cologne, [3]University of Duisburg-Essen

The vermiform adult Bauplan is universally conserved in nematodes. This is contrasted by inter-species variations on a morphological, and genetic level occurring throughout early embryonic development. For instance, different arrangements of blastomeres and the exchange of key developmental genes have been observed. In spite of differences on the genetic level, nematode species pass through common developmental stages, marked by changes in gene expression.

Here, we investigate two species with different degrees of relatedness to the model species *Caenorhabditis elegans*. *Romanomermis culicivorax* is very distantly related to *C. elegans*, and its genome encodes many genes important for development in other bilaterians but not found in *C. elegans*. Conversely, it lacks genes important for *C. elegans* development, demonstrating variability of developmental processes in nematodes. *Panagrolaimus sp*. PS1159 is more closely related to *C. elegans*, and its development is very similar on a morphological level. However, some key genes known to play a role in the regulation of *C. elegans* axis formation are not encoded in its genome, suggesting that differences at the level of genetic regulation can occur even in species where the processes themselves seem to be following the same patterns.

We collected 120 embryos of different stages for each species throughout embryonic development. Using the CEL-Seq2 proto-

col, we sequenced single-embryo transcriptomes and compared gene expression divergence between species following embryonic development. We further analysed the gene expression on a single-cell level via single-cell sequencing for the 1-cell and 2-cell stage in *Panagrolaimus sp*. PS1159, which are the stages in which axis formation is determined in *C. elegans*.

The comparison of nematode developmental time courses, displaying gene expression divergence, is a starting point to examine limiting effects of development on evolution that could explain the conserved vermiform adult Bauplan. The assessment of gene expression at comparable stages in different species further allows us to investigate the flexibility of developmental regulation through genetic control.

490A **GWA in a bottle: a flexible experimental system for genome-wide association mapping in *C. elegans* and related nematodes** Stefan Zdraljevic[1,2,3], Laura Walter-McNeill[1,2,3,4], Heriberto Marquez3[1,2,3], Amy K Webster[5], Rojin Chitrakar[6,7], Ryan L Baugh[6,7], Joshua S Bloom[1,3,3], Leonid Kruglyak[2,3,81]Human genetics, University of California, Los Angeles, [2]Howard Hughes Medical Institute, [3]Biological Chemistry, University of California, Los Angeles, [4]Human genetics, UCLA, [5]Institute of Ecology and Evolution, University of Oregon, [6]Center for Genomic and Computational Biology, Duke University, [7]Biology, Duke University, [8]University of California, Los Angeles

A central goal of modern genetics research is to identify DNA sequence variants that contribute to phenotypic variation. Growing catalogs of sequenced genetically diverse isolates enable genome-wide association (GWA) studies of phenotypes of interest. However, husbandry and phenotyping of many individual isolates is time-consuming and laborious. One approach to address this problem is bulk competition of isolates in relevant conditions, with molecular inversion probes (MIPs) used to track the frequency of each isolate over time. However, the flexibility of this approach is limited because MIPs must be carefully designed and validated to target a variant unique to each isolate in the pooled population. The existing MIP library in *C. elegans* covers only a fraction of cataloged isolates.

Here, we present a whole-genome sequencing (WGS) approach that leverages the existing catalog of variants in a set of isolates. We developed computational methods to infer the frequency of each isolate present in a pooled population from low-coverage WGS data. We used simulations to show that this approach can accurately infer the frequencies of all 550 wild *C elegans* strains in the *C. elegans* Natural Diversity Resource (CeNDR) with as little as 10x WGS coverage per pooled experiment. Next, we compared strain frequencies inferred with the WGS approach to those measured using MIP libraries. We acquired WGS data for samples that contain 100 distinct *C. elegans* strains. These samples came from a previously published experiment that used a MIP library to measure strain frequencies. With fewer distinct strains in the pooled population, we found that 1x WGS depth was sufficient to accurately recapitulate the strain frequencies obtained using the MIP-library approach.

To facilitate GWA mapping experiments using pooled populations, we constructed and cryogenically froze eight sets of ~48 strains from the CeNDR collection. We next pooled these eight strain sets together to generate a mapping population of 330 strains. This approach provides the flexibility to extend the mapping population as new strains become available through CeNDR. WGS-based inference of strain frequencies is generalizable to other species that can be clonally propagated.

491A **Marvelous Mutants of C. *inopinata*: Forward Screen Reveals Body Size Mutations** Kimberly Moser, Gavin WoodruffBiology, University of Oklahoma

Body size is a fundamental organismal trait varying widely among species. *Caenorhabditis inopinata* grows to be nearly twice as long as its close relative, *C. elegans*. Because of its relationship to this model system, *C. inopinata* is well-positioned to address the causes of body size variation within a comparative molecular genetics context. Here, we report a pilot forward mutagenesis screen to discover genes underlying body size in this species.

We screened 493 mutagenized haploid genomes for recessive body size mutations in the F_3 generation (as *C. inopinata* is a gonochoristic species). We established five mutant homozygous lines after backcrossing for five generations to purge background mutations. Three of these lines harbor a short and fat (dumpy) phenotype, whereas two of these lines have a long mutant phenotype.

Currently we are characterizing mutants to estimate effects on body size dimensions, rates of growth, and the onset of body size differences. Bulk segregant analyses with mutant and wild-type F_2 individuals are also underway to pinpoint the molecular lesions that underlie these mutant phenotypes. Once identified, genes critical for body size regulation in *C. inopinata* can be compared with homologous genes in *C. elegans* using molecular and developmental genetic approaches. This, in tandem with further forward screens, will reveal the extent of functional evolution of body size genes in species with exceptional body sizes.

492A ***F1 hybrid male sterility can be rescued by homozygotic X-chromosome introgression*** Yiwen ZHANG, Yanwen Shao, Runsheng LiCity University of Hong Kong

Hybrid male sterility is one of the most common hybrid incompatibilities (HI) to prevent gene flow between species. The cross between *Caenorhabditis. briggsae* male and *Caenorhabditis. nigoni* female can only produce sterile F1 males. By incorporating different *C. briggsae* X chromosome fragments into *C. nigoni* background, we are able to produce a homozygotic introgression strain (ZZY10253), which could mate with *C. briggsae* male and produce fertile F1 males. The introgression region contains 2.2 Mb C. *briggsae* X chromosome. After multiple rounds of backcrossing of ZZY10253 with *C. nigoni*, the introgression fragment can be further reduced to 1.7 Mb (500 Kb shorter). The mating of *C. briggsae* male and this strain (ZZY10254) can only produce sterile F1 males, similar to wild-type *C. nigoni* worms. The finding indicates that the 48 genes inside the 500 Kb interval could contain the key genes for F1 male sterility.

We performed RNA-seq and small RNA-seq for the male worms from ZZY10253, ZZY10254, the fertile F1 male hybrids from the crossing between *C. briggsae* male and ZZY10253 female (F1[B,253]), and the sterile F1 male hybrids from the crossing between *C. briggsae* male and ZZY10254 female(F1[B,254]),. We observed the up-regulated of autosomal-related spermatogenesis genes in F1[B, 253], indicating an X-autosomal interaction. We tried to narrow down the gene locus responsible for F1 hybrid male sterility using RNA-seq and small RNA-seq. We have located three loci for further validation.

493A **Co-segregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode** Caroline Blanc, Nathanaelle Saclier, Ehouarn Le Faou, Sylvain Glemin, Nicolas Galtier, Marie DelattreCNRS

In asexual animals, female meiosis is modified to produce diploid oocytes. Associated with recombination, this is expected to lead to a rapid loss of heterozygosity, with adverse effects on fitness. Many asexuals, however, keep a highly heterozygous genome, the underlying mechanisms being most often unknown.

We show that in the nematode Mesorhabditis belari, asexual females evolved a unique form of meiosis with recombination, whereby either the two recombinant or the two non-recombinant chromatids of a given pair of homologs are transmitted from mother to daughter. This segregation bias, which we call Directed Chromatid Assortment, avoids the adverse effects of heterozygosity loss.

We demonstrate this novel mechanism via a combination of cytological, genomic and theoretical approaches. By visualizing the successive steps of meiosis and distinctively marking the two chromatids of meiotic chromosomes, we show that homologous chromosomes recombine at each meiosis, fail to segregate during meiosis I, but undergo a biased segregation during meiosis II, during which recombinant chromatids co-segregate. Genomic data confirmed a high rate of recombination and demonstrated that heterozygosity is preserved from mother to daughter and at the population level. A theoretical model confirmed that this segregation bias is necessary to account for the observed pattern and likely to evolve under a wide range of conditions.

Our study uncovers an unprecedented case of non-Mendelian genetics, opening a wide range of questions on its mechanistic basis and evolutionary implications.

494A **Getting inside sperm gigantism: Electron microscopy of *C. macrosperma*'s giant sperm cells** Rebecca Schalkowski, Asher D. CutterEcology & Evolutionary Biology, University of Toronto

Sperm cell gigantism has evolved repeatedly across the *Caenorhabditis* phylogeny, including in *C. macrosperma*. To investigate whether these unusually large gametes contain an unusual complement of intracellular features, we have conducted comparative electron microscopy of sperm cells from *C. macrosperma* and *C. nouraguensis*, a close relative with standard sperm cells. We hypothesized that sperm might differ in the density of mitochondria if energetic demands on sperm motility or longevity influence selection on sperm cell size. We also hypothesized that sperm might differ in the density of membranous organelles (MOs) if the volumetric contribution of MO contents to the ejaculate provides an important source of selection on sperm cell size. TEM cross-sections of mature spermatozoa inside mated females affirms size differences between species, as well as greater total area corresponding to mitochondria and MOs in *C. macrosperma*. Scaled for non-pseudopod cell area, however, mitochondria show only a small trend toward greater contribution to cell size for *C. macrosperma* sperm. Scaled to the cross-sectional length of the cell perimeter, MOs tend to comprise a somewhat smaller component of *C. macrosperma* sperm cell membrane. These observations suggest that selection leading to differences in sperm cell size are unlikely to be strongly related to mitochondrial demands or MO contents, with an untested alternative hypothesis being that selection on sperm cell size might instead be mediated primarily by biophysical demands on pseudopod adhesion.

495A **Evo-Devo-Neuro: Neuronal specification by terminal selectors in the *Pristionchus pacificus* nervous system** Curtis Loer[1], Yasmin H Ramadan[2], Steven J Cook[2], Hanh Witte[3], Ralf Sommer[3], Oliver Hobert[21]Biology, Univ San Diego, [2]Biol Sci, Columbia Univ, [3]Evol Biol, Max Planck Inst for Dev Biol

The nervous systems of many nematodes are remarkably similar despite considerable divergence of their genomes. To exam-

ine nematode nervous system evolution, we are characterizing the nervous system of *Pristionchus pacificus (Ppa)* to compare with that of *C. elegans (Cel)*. To study neuronal specification in *Ppa,* we have epitope-tagged transcription factor (TF) genes to examine their expression patterns, including *Ppa* orthologs of *unc-3*, *unc-86* and *unc-42*, and generated deletion mutants for each gene. [An antiserum to *C. elegans unc-86* (Finney & Ruvkun, 1990) works in *Ppa,* showing a pattern identical to that of the epitope-tagged locus; staining is absent in *Ppa-unc-86* mutants, confirming specificity of the antiserum.] These TFs are known in *C. elegans* to act as 'terminal selectors' – TFs that initiate and maintain neuronal identity by regulating batteries of terminal differentiation genes such as those required for using a specific neurotransmitter (e.g., Hobert, 2016; Curr Top Dev Biol 116: 455). Expression patterns of *unc-3*, *unc-86* and *unc-42* genes in *Ppa* appear to be quite similar to those in *Cel,* but not identical. Changes in serotonin (5HT) expression in *Ppa-unc-86* suggest both similar and different roles in *Ppa*. For example, NSMs require *unc-86* to express 5HT in both *Cel* and *Ppa*; unlike in *Cel*, body wall neurons frequently express 5HT in *Ppa-unc-86* mutants, suggesting cell fate transformations. Like in *Cel*, expression of cholinergic proteins in the ventral & dorsal nerve cords is reduced or absent in *Ppa-unc-3* mutants. We will present these and other results of our analyses of these neuronal specification TFs. We have also examined expression of the homeodomain TF *ceh-48,* which in *Cel* is pan-neuronally expressed. Our analyses indicate that *Ppa-ceh-48* is also pan-neuronal; for example, the number and pattern of *ceh-48*-positive nuclei in the head of *Ppa* matches that of EM-defined neuronal nuclei in serial section reconstructions used to determine the *Ppa* head connectome. We can therefore fully define the nervous system of *P. pacificus* – numbers and locations of all neurons.

496A **Rewiring TRA-1 regulation in germ cells during nematode evolution** Yongquan Shen[1], Shin-yi Lin[1], Ronald E Ellis[21]Rowan University SOM, [2]Molecular Biology, Rowan University SOM

The transcription factor TRA-1 is the sole nematode Gli protein. Because TRA-1 is the master regulator of sexual identity, it is subject to complex regulatory interactions, some of which played key roles in the evolution of self-fertility. One factor is the FEM complex, which ubiquitinates TRA-1, leading to its elimination in *XO* males. We are focusing on the other regulator of TRA-1 — its direct interaction with a fragment of the TRA-2 receptor. This interaction is disrupted by the *mx* alleles in *C. elegans,* which result in *XX* animals developing as females rather than as hermaphrodites. How this interaction controls germ cell sex has been a mystery for forty years.

Three lines of evidence imply that *C. elegans* TRA-1 sequesters TRA-2 in the germ line to promote spermatogenesis. First, reducing *tra-1* activity increases the probability germ cells will develop as oocytes, which suggests that TRA-1 negatively regulates TRA-2 (Wang & Kimble 2001). Second, the *tra-2(mx)* alleles cause a gain-of-function in gene dosage studies, which implies that the loss of negative regulation increases TRA-2 activity. Third, the effect of these *mx*alleles is sensitive to levels of the FEM proteins, showing that they increase the ability of TRA-2 to target FEM-3.

These analyses in *C. elegans* are challenging, because the three *fem* genes are absolutely required for spermatogenesis. However, that is not the case in *C. briggsae.* Thus, we produced orthologous *Cbr-tra-1(mx)* alleles by gene editing. As in *C. elegans,* these mutations disrupt the TRA-1/TRA-2 interaction. Surprisingly, the *cbr-tra-2(mx)* mutants increase the number of self progeny, indicating that they *increase* hermaphrodite spermatogenesis, rather than eliminate it. Furthermore, these mutations also restore spermatogenesis to *cbr-she-1(v35) XX* females. Similarly, the *tra-1(v197v383)*allele also disrupts the interaction with TRA-2, increases spermatogenesis and suppresses *she-1*. Crucially, these effects do not require *fem-3* in *C. briggsae.*

Thus, we propose that *C. briggsae* SHE-1 regulates TRA-2, which in turn acts directly on TRA-1 to control germ cell fates. This specification of spermatogenesis depends on TRA-1 forming a full-length Gli activator, since it is blocked by the *tra-1(v48)* mutation, which eliminates activator function. Hence, we infer TRA-2 binds TRA-1 to block Gli activation function. By contrast, in *C. elegans* the primary effect of TRA-2/TRA-1 binding is to favor spermatogenesis by sequestering TRA-2 and prevent it from acting on the FEM complex, which acts downstream of TRA-1.

Thus, the flow of information in this Gli transcription pathway has changed dramatically during recent evolution.

497A **Investigating P granule localization and temperature dynamics across the *Caenorhabditis* phyla** Lisa PetrellaBiological Sciences, Marquette University

We are interested in how changes in P granule composition, structure, and dynamics across *Caenorhabditis* species impact the thermal limits of fertility. P granule properties have been studied extensively in *C. elegans*, where the presence of P granules is critical for fertility at elevated temperatures. P granules form through liquid-liquid phase separation within the cytoplasm. This process is subject to dissolution at elevated temperatures, possibly explaining the correlation between P-granule function and sterility observed in *C. elegans* at elevated temperatures. Two related *Caenorhabditis* species, *C. briggsae* and *C. tropicalis,* have higher temperature fertility limits of a 30-31°C, unlike *C. elegans,* which goes sterile between 26-27°C. To determine if part of the thermal shift in fertility limits in these species correlates with the changes in dissolution of P granules at higher tempera-

tures, we used CRISPR-Cas9 to GFP-tag the conserved DEAD-box helicase GLH-1 homologs in these species. We then tested the disillusion of GLH-1::GFP-containing granules in the oocytes of all three species when incubated at 31°C for 30 min and found that GLH-1::GFP remains much more granular in both *C. briggsae* and *C. tropicalis.* When studying the localization of GLH-1::GFP containing P granules, we found that in the distal germline GLH-1::GFP localization patterns look similar across the three species, but that there are distinct differences in GLH-1::GFP localization in oocytes and embryonic P cells in the three species. Specifically, in both *C. briggsae* and *C. tropicalis,* P granules stay perinuclear longer in oocytes and become perinuclear earlier in embryonic development than *C. elegans.* Since previous work has shown that perinuclearly located P granules tend to be more stable than cytoplasmic P granules, these changes in localization alone could have profound effects on P granule dynamics at elevated temperatures. Using these closely related species will allow us to further understand the evolution of P granule composition, structure, and dynamics by doing domain swapping of crucial P granule components to further understand the potential correlation between P granule dissolution and fertility. These investigations provide a new model for understanding fertility loss under temperature stress as a function of the changes in physical properties of intracellular structures that can evolve to provide different temperature fertility limits.

498B **Nigon elements as organising principles in nematode genome evolution** Mark Blaxter[1], Erna King[2], Lewis Stevens[2], Pablo Gonzalez de la Rosa[2], Manuela Keininger[2], Christopher Laumer[31]Wellcome Sanger Institute, [2]Tree of Life, Wellcome Sanger Institute, [3]Natural History Museum

The karyotype of Caenorhabditis elegans – five autosomes and an X chromosome – is also found in other species in the genus, but across Nematoda karyotypes vary widely, with chromosome counts ranging from 2 to over 50. We are interested in how nematode karyotypes evolve, and how both stability and dynamic evolution pattern the genome. We previously inferred a set of seven ancestral linkage groups – named Nigon elements – and described the origin of the extant karyotypes of nematode species from Rhabditina (Clade V), Tylenchina (Clade IV) and Spirurina (Clade III) through processes of fusion, fission and mixing of these elements.

Inference of the pattern and process of evolution of karyotypes from genomic data requires high quality genome sequences assembled to chromosomal completeness. Genomes of this quality were previously available for only a few species of nematode. We have been working to generate chromosomally-complete assemblies for a wide range of nematodes using accurate long read and chromatin conformation capture data. For many species we have used inbred strains grown in culture, but this option is not possible for most nematodes. We have therefore deployed a recently-developed picomolar-input multimodal sequencing technique that generates long read data from single nematode specimens. Assemblies derived from this single-specimen data can then be scaffolded using conformation capture data derived from pooled specimens.

Using these approaches we have generated genome assemblies of over 30 parasitic and free-living members of Rhabditina, as well as of members of previously unsampled orders of Chromadoria and Enoplea. Mapping the genes used in defining Nigon elements to these assemblies, we have discovered the first species where all seven elements are present as distinct chromosomes (Allodiplogaster pararmata) and better resolved the processes that have generated extant rhabditid karyotypes. Exploring genomes from species across the phylum, we find that the Nigon elements are not conserved, and thus conclude that Nigon elements are ancestral linkage groups of Rhabditida.

499B **What can we learn about aging from an experimental population system with *C. elegans*?** Andrea Scharf Biological Sciences, Missouri University of Science and Technology

Age-related changes and death of individual organisms are extensively studied in laboratories, but we have only limited knowledge on population and ecosystem levels. Old individuals exist in wild populations; however, many wild populations seem not to support the survival of aged seniors. What are the factors that determine whether a population supports older animals and allows individuals to die of old age?

We developed an experimental population system based on *C. elegans* that allows us to track and manipulate populations over months. To complement this experimental system, we developed an agent-based model to simulate the laboratory worm ecosystem and to conduct experiments that are impossible to do in reality. We used these platforms to investigate the conditions that permit *C. elegans* in a population to die of old age, a critical step in understanding the role of aging in population dynamics.

We discovered that maximum lifespan, rate of adult culling, and progeny number/food stability control whether a population would permit old age as a cause of death. In more detail, populations displayed a tipping point for aging as the primary cause of adult death. Adults died young in populations with high progeny numbers, while a slight decrease in progeny number caused a dramatic shift in the population, and adults survived to old age. The conditions defined here establish a conceptual framework for understanding why certain animals die of old age in the wild including mayflies and elephants.

1. Scharf, A., Mitteldorf, J., Armstead, B., Schneider, D., Jin, H., Kocsisova, Z., Tan, C., Sanchez, F., Brady, B., Ram, N., DiAntonio, G.B., Wilson, A.M., and Kornfeld, K. A laboratory and simulation platform to integrate individual life history traits and population dynamics. Nat Comput Sci 2, 90–101 (2022). https://doi.org/10.1038/s43588-022-00190-8

500B **Studying the mode of action of the new nematicide cyclobutrifluram in *Caenorhabditis elegans*** Fariba Heydari[1], David Rodriguez Crespo[1], Chantal Wicky[21]Biology, University of Fribourg, [2]Biology, University of Fribourg, Switzerland

World agriculture is challenged with infections mediated by plant parasitic nematodes (PPNs). These infections lead to great economic loss and compromise food supply. For a long time, management of PPN infections has relied on nematicides that impact not only parasitic nematodes, but also other organisms. Cyclobutrifluram belongs to a new category of nematicides that appear to target PPNs more specifically, however, its molecular mode of action is still unknown. Using *Caenorhabditis elegans*, we demonstrated here that cyclobutrifluram impacts the nematode survival rate and its reproduction. Cyclobutrifluram treated worms show a decreased germ cell proliferation rate and an increased level of apoptosis. Furthermore, we could show that two mutant strains, that exhibit missense mutation in the gene coding for the mitochondrial succinate dehydrogenase (SDH), are resistant to cyclobutrifluram treatment. This result allows us to propose that cyclobutrifluram nematicide effect relies on the inhibition of the mitochondrial complex II.

Additionally, we performed a transcriptomic analysis in order to identify which metabolic pathways are perturbed upon cyclobutrifluram treatment. We observed that genes encoding detoxifying proteins, such as cytochrome P450s and UDP-glucuronosyl transferases (UGTs), are highly deregulated in cyclobutrifluram exposed worms. Overall, these results confirm that *C. elegans* is a suitable model organism to study the mode of action of nematicides. They also contribute to increase our understanding of nematicide activity, and allow a better management of potential resistance to nematicide treatments.

501B **Substantial Programmed DNA Elimination in Mesorhabditis nematodes** Carine Rey, Caroline Launay, Eva Wenger, Marie DelattreCNRS

More than 150 years ago, Theodor Boveri described for the first time Programmed-DNA Elimination (PDE) in the parasitic nematode *Parascaris univalens*, where chromosomes are highly fragmented and partially destroyed in all somatic cells in early embryos. Adults thus have an intact germline genome and a reduced genome in the soma. Since then, the same phenomenon has been observed in other animals from very distinct phyla. PDE raises fascinating questions related to DNA repair, genome stability, repeated elements, germline maintenance, and genome evolution. Yet, the ultimate function and the mechanisms of PDE remain mysterious in animals, because the species that have described so far are difficult to study and not amenable to functional approaches.

Through cytological observations, we have fortuitously discovered substantial Programmed DNA Elimination in a genus of free-living nematodes, *Mesorhabditis*, within Rhabditina (where *C. elegans* also belong). These worms can be handled like *C. elegans* and we have established RNAi and CRISPR/Cas9. Hence, *Mesorhabditis* species offer, at last, an opportunity to develop functional approaches for the study of a long-lasting question in biology.

We have described the cytological events leading to PDE in the soma of *Mesorhabditis belari*. We have also performed a comparative genomic approach which revealed that although two *Mesorhabditis* species eliminate repeated elements and few protein coding genes, the targeted genes are very different. They are also poorly conserved overall, demonstrating that PDE does not target key developmental genes (Rey & al., BioRxiv). We will present our recent results on the identification of breakpoint regions and our screen to identify the molecular machinery involved in PDE.

Concomitant work performed initially by the Blaxter lab (Gonzalez de la Rosa & al., G3, 2021), and recently by the Wang lab (Dockendorff & al., CurrentBiol, 2022), has identified PDE in another species within Rhabditina, *Oscheius tipulae*, although here, elimination concerns only chromosome ends. We systematically looked for PDE in ~40 other non-parasitic nematode species within Rhabditina and Tylenchina using DNA staining and DNAFISH. We discovered that PDE is more pervasive than anticipated in nematodes.

502B **COSMIC signature and strand symmetry of spontaneous mutations in *Caenorhabditis elegans*** Moein F Rajaei, Charles F BaerBiology, University of Florida

Mutation is the fuel of evolution, as well as the underlying cause of many diseases, including cancer. Particular sources of mutation often have a characteristic signature, defined as the unique combination of mutation types that are related to a specific mutagenic process. We previously demonstrated that the frequency distribution of type-specific mutations (the “mutational spectrum”) differs between mutations accumulated in laboratory “mutation accumulation” (MA) lines and wild isolates of the nematode *C. elegans*, but that the difference is largely restricted to mononucleotide repeats. By using The Catalogue Of Somatic

Mutations In Cancer (COSMIC) database, we reanalyzed the sequence data to characterize the mutational signature of 7,053 base-substitution variants accumulated in three different sets of mutation accumulation lines under relaxed selection and also the signature of rare variants in a set of wild isolates, toward the goal of understanding the underlying sources of variation in the mutational process.

The predominant COSMIC signature in non-mononucleotide sequence is SBS40 in both MA lines and wild isolates. The proposed etiology for SBS40 is "unknown". In contrast, SBS90 is the primary signature in mononucleotide regions of MA lines, whereas it was not identified in rare standing variants. The proposed COSMIC etiology for SBS90 is exposure to Duocarmycin, a natural product first isolated from Streptomyces bacteria. Duocarmycin alkylates DNA, and although the MA lines did not encounter Streptomyces, many DNA damaging agents may present a similar spectrum of mutations.

We also examined the same set of mutations in MA lines and wild isolates for strand asymmetry, which distinguishes whether the mutations occurred on the forward or reverse strand. Strand asymmetry has several potential causes, including association with transcription (transcription coupled repair or mutagenesis) and differential mutability of the leading vs. the lagging strand during replication. Our initial analyses identified no significant strand asymmetry in either MA lines or wild isolates.

503B **Direct inference of the distribution of fitness effects (DFE) of spontaneous mutations from recombinant inbred *C. elegans* mutation accumulation lines: support for Fisher and Gillespie** Timothy A Crombie[1], Moein F Rajaei[2], Ayush S Saxena[2], Lindsay M Johnson[2], Sayran S Saber[2], Robyn S Tanny[1], Erik C Andersen[1], Jose Miguel S Ponciano[2], Charles F Baer[21]Molecular Biosciences, Northwestern University, [2]Biology, University of Florida

The distribution of fitness effects (DFE) of new mutations is a fundamental parameter in evolutionary biology, and has practical application in the context of modeling the genetic basis of complex heritable disease. However, the DFE is very difficult to estimate empirically. At present, nearly all estimates of the DFE rely on indirect statistical inference, either from the standing site-frequency spectrum or from laboratory estimates of fitness in mutation accumulation (MA) lines, with no direct connection between the underlying mutations and their effects on fitness. Here, we report results from a set of ~500 recombinant inbred lines (RILs) derived from a cross between two *C. elegans* mutation accumulation (MA) lines. The RILs segregate 169 mutations (SNPs and small indels), with little linkage disequilibrium beyond ~1 Kb. We assayed the set of lines for competitive fitness against a reference strain. Three results stand out. First, the set of mutations collectively explain about 60% of the broad-sense heritability (i.e., the among-line variance; H^2~18%), and a model in which the mutational effect is constrained to equal 0 is significantly less likely. Thus, there is significant variation in the DFE. Second, the DFE is roughly symmetric around zero, with a perhaps surprisingly large fraction of mutations with positive effects on fitness. However, there was a marginally-significant ($P<0.08$) trend for mutations with larger absolute effects to be more likely to have negative effects, i.e., to be deleterious. These findings are broadly consistent with R. A. Fisher's Geometric Model of evolution, and also with John Gillespie's critique of the Nearly Neutral Theory of molecular evolution, which he argued predicts that a large fraction of weakly-selected variants must be beneficial.

504B **The *C. elegans* proteome response to two protective *Pseudomonas* symbionts** Barbara Pees, Lena Peters, Christian Treitz, Andreas Tholey, Katja DierkingUniversity Kiel

The two *C. elegans* natural microbiota isolates *Pseudomonas lurida* MYb11 and *Pseudomonas fluorescens* MYb115 protect the host against pathogens through distinct mechanisms. While *P. lurida* produces an antimicrobial compound and directly inhibits pathogen growth, *P. fluorescens* MYb115 protects the host without affecting pathogen growth. It is unknown how these two protective microbes affect host biological processes. We used a proteomics approach to elucidate the *C. elegans* response to MYb11 and MYb115. We found that both *Pseudomonas* isolates increase vitellogenin protein production in young adults, which confirms previous findings on the effect of microbiota on *C. elegans* reproductive timing. Moreover, the *C. elegans* responses to MYb11 and MYb115 exhibit common signatures with the response to other vitamin B12-producing bacteria, emphasizing the importance of vitamin B12 in *C. elegans*-microbe metabolic interactions. We further analyzed specific signatures in the *C. elegans* response to MYb11 and MYb115. We provide evidence for distinct modification in lipid metabolism by both mutualistic microbes. We could identify activation of host pathogen defense responses as MYb11-specific proteome signature and demonstrate that the intermediate filament protein IFB-2 is required for MYb115-mediated protection. These results indicate that MYb11 not only produces an antimicrobial compound, but also activates host antimicrobial defenses, which together might increase resistance to infection. In contrast, MYb115 affects host processes such as lipid metabolism and cytoskeleton dynamics, which might increase host tolerance to infection. Overall, we provide new insights into the potential mechanisms underlying *C. elegans* microbiota-mediated protection from pathogen infection.

505B **The wild microbes of a fig worm** Gavin Woodruff Biology, University of Oklahoma

Caenorhabditis nematodes eat microbes. As there are over 70 known *Caenorhabditis* species, variation in microbial environments and diets may be important drivers of divergence in this group. To address this, we are characterizing the natural microbes

associated with *C. inopinata*, a fig-associated close relative of *C. elegans*. In 2019, 38 *Ficus septica* figs (across 12 plants in Taiwan) were dissected. Metadata such as foundress wasp number and nematode occupancy (among others) were collected for each fig. Suspensions derived from interior fig material (as well as fig surface washes taken before dissection) were prepared for 16S microbial metabarcoding. Over 3,000 OTU›s were detected, and microbial communities were dominated by members of *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota*. Although microbial communities of fig exteriors and interiors could be distinguished, levels of microbial diversity were comparable across these areas of the fig. No differences in the composition or diversity of microbial communities were detected among figs with or without nematodes. Despite this, a handful of OTU's (associated with the genera *Kosokonia*, *Ochobactrum*, and *Stenotrophomonas*) revealed differential abundance among figs varying in nematode occupancy. And although nematodes were more commonly found in figs with more fig wasp foundresses, foundress number had no detectable impact on microbial community composition. Future work will interrogate nematode and wasp communities specifically; we are also characterizing nematode-microbe interactions through laboratory experiments. Taken together, these results constitute a fundamental step in characterizing the natural microbial universe of *Caenorhabditis* nematodes.

506B **Evolution and plasticity of *Caenorhabditis* egg-laying behaviour** Clotilde GIMOND[1], Laure Mignerot[1], Lucie Bolelli[2], Charlotte Bouleau[3], Asma Sandjak[2], Christian Braendle[21]Institut de Biologie Valrose, CNRS, [2]CNRS, [3]CNRS/UCA

C. elegans egg-laying behaviour underlies a structurally simple neural circuit, which has served as an important model in neurogenetics. Here we present our ongoing characterization of natural divergence in the nematode egg-laying circuit, ultimately aimed at identifying the neural and molecular determinants that generate variation in this central reproductive behaviour. Analysing ~40 *Caenorhabditis* species and hundreds of wild isolates, we show that the nematode egg-laying circuit exhibits complex evolutionary variability, not only among populations within *C. elegans* but also among different *Caenorhabditis* species. Species and isolates also differ strongly in egg-laying activity in response to diverse neuromodulatory agents and sensory stimuli, suggesting that these differences arise through adaptation to distinct ecological niches.

507B **Predator-prey coevolution drives natural diversity in *Caenorhabditis elegans* chemotaxis towards predatory fungal odor** TzuHsiang Lin[1,2,3], Han-Wen Chang[1,4], Ching-Han Lee[1,4], Ching-Ting Yang[1], Yu-Hsun Huang[1], Yu-Shi Chiang[1], Erik C. Andersen[5], Yen-Ping Hsueh[1,2,41]Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan, [2]Genome and Systems Biology Degree Program, Academia Sinica and National Taiwan University, Taipei 10617, Taiwan, [3]Department of Life Science, National Taiwan University, Taipei, 10617, Taiwan, [4]Molecular Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan, [5]Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA

Predator-prey interactions are ubiquitous across the tree of life. These interactions often lead to behavior changes that promote survival, but the molecular mechanisms underlying behavioral evolution remain unclear. Using the model nematode *Caenorhabditis elegans* and its natural predator *Arthrobotrys oligospora*, we studied the chemotaxis behavior toward the attractive odor produced by *A. oligospora*, methyl 3-methyl-2-butanoate (MMB). We found that this chemotaxis behavior exhibits natural diversity with ecological significance. Specifically, a wild Taiwanese *C. elegans* strain (TWN2542) was collected from the same sample as the nematode-trapping fungus *A. oligospora*, and this strain has low attraction to MMB. Using bulk-segregant analysis, we mapped this trait to a 2.4 Mb quantitative trait locus (QTL) on chromosome IV with a haplotype shared with six other low MMB chemotaxis wild strains. However, our candidate approach targeting G protein-coupled receptors potentially involved in MMB reception failed to reveal any causal genes. We then narrowed the QTL by generating near-isogenic lines that contain hyper-divergent regions in this genomic region. This information will allow us to identify putative mechanisms involved in MMB chemotaxis and investigate the evolutionary processes that underlie genetic variation among natural populations of *C. elegans*.

508B **Linking wild alleles to acute ethanol behavioral responses in *C. elegans*** Marijke H. van Wijk[1], Elizabeth C. Quamme[2], Andrew G. Davies[2,3], Joost A. G. Riksen[1], Mark G. Sterken[1], Jan E. Kammenga[1], Jill C. Bettinger[2,31]Wageningen University & Research, [2]Virginia Commonwealth University, [3]Virginia Commonwealth University Alcohol Research Center

Alcohol use disorder is a major problem worldwide and is for 50% determined by genetics. A reliable genetic predictor of alcohol use disorder risk is the acute physiological response to alcohol. Identifying genes and pathways that modulate acute ethanol response behaviors is therefore essential to understand the molecular underpinning of alcohol use disorder. Acute ethanol responses are conserved across species, including nematodes. Here, we leveraged natural variation in *C. elegans* to link naturally occurring wild alleles to acute ethanol response behaviors. We behaviorally characterized multi-parent recombinant inbred lines in an ethanol response locomotion assay. We exposed animals to 0 mM, 200 mM, or 400 mM exogenous ethanol and tracked locomotion at 10, 30, and 50 minutes of continuous exposure. We found that the phenotypes: initial sensitivity (effect of 400 mM ethanol at 10 min), acute functional tolerance (recovery from locomotor depression between 10 and 30 minutes on 400 mM ethanol), and low concentration locomotor activation (faster speed relative to untreated at 30 minutes of exposure to 200 mM ethanol) had narrow sense heritabilities of 0.28, 0.13, and 0.36, indicating that the observed phenotypic variance can partly be explained by additive genetics. To statistically link alleles to phenotypic variance we mapped QTL. We found two QTL

for low concentration locomotor activation that together explain 45.5% of the observed phenotypic variance in this population. We will validate these QTL using introgression lines in which the genetic background consists of JU1941 and the QTL loci comes from JU1931. Further investigations in narrowing the confidence interval and candidate gene prioritization will hopefully lead to identification of genes that modulate low concentration locomotor activation in wild nematode populations.

509B **Interrogating the evolution of host-microbe interactions with fig worms** Austin Link[1], Gavin C. Woodruff[21]University of Oklahoma, [2]Biology, University of Oklahoma

Animals live in a microbe-rich world, and host-microbe interactions influence fitness and health. *Caenorhabditis* nematodes have been a biomedical model system for decades. Laboratory cultures of such animals are reared on *Escherichia coli* bacteria for food. As rotting plant bacterivores, this is an ecologically artificial environment implemented for experimental convenience. Only recently has the natural microbial context of these organisms been considered. How can natural microbial associates inform the biology of longstanding experimental systems? To understand the evolution of host-microbe interactions, we have isolated forty-five strains of wild microbes associated with the nematode *C. inopinata* in nature. *C. inopinata* is the closest known relative of *C. elegans* and is associated with figs and their pollinating wasps. Here, we aim to rear *C. inopinata* on these isolates and measure nematode fecundity, growth rates, and other life history traits to discover how microbes in its natural context impact nematode fitness. We also aim to perform similar experiments with *C. elegans* to describe how host-microbe interactions evolve. So far, we have found that one strain, from the genus *Routella*, resulted in a one hundred twenty-seven percent mean increase of fecundity compared to *Escherichia coli*. Once more of these interactions are characterized, we will implement forward mutagenesis screens to discover the genes (in worms and microbes) important for microbe-dependent fitness effects. In this way, we will use ecologically relevant laboratory contexts to unearth novel functions of unexplored genes and understand the genetic bases of host-microbe interactions.

510B **A hybrid non-motile cilium requires motility apparatus for mechanosensation** Dhruvin akbari, Diako Nazari, Samantha Zhou, Michel LerouxMolecular Biology and Biochemistry, Simon Fraser University

As eukaryotes evolved from single-celled organisms to metazoans, the motile cilium played a crucial role in powering cell movement and generating fluid flow. With the emergence of multicellularity, cell-specific transcriptional programs endowed cells with specialised functions. As a result, the need for motile cilia in every single cell ceased, which allowed some of the cells to re-purpose their cilia into non-motile, strictly sensory organelles. Since then, both types of cilia, motile and non-motile, co-existed in most multicellular organisms. However, nematodes like *C. elegans* lost motile cilia during their evolutionary history, retaining only sensory cilia.

While virtually all cilium motility genes were discarded from nematode genomes, we discovered that several motility-associated genes remain. These include human homologues of Inner Dynein Arms (IDA) heavy, intermediate, and light intermediate chains, and an IDA assembly factor; a Radial Spoke gene is also present. From single-cell transcriptome studies, all motility cilium-associated genes appear to be specifically transcribed in the Outer Labial Quadrant (OLQ) class of ciliated neuron, which is mechanosensory and responsible for nose-touch sensation and foraging behaviour. We hypothesise that the encoded proteins were retained in nematodes to form a functional module that performs a mechanosensory role within OLQ cilia. Why these particular proteins were retained to fulfill such a function is unclear. We speculate that in motile cilia, the IDA proteins may play related roles in sensing the position of the organelle during its movement (a form of 'proprioception'), which is necessary for regulating the precise waveform of the motile cilium.

By CRISPR-mediated endogenous GFP tagging of the relevant genes, we found that the proteins localise specifically to the OLQ cilia in the head of the animal. Moreover, we determined that the IDA intermediate chain depends on the IDA light intermediate chain for its OLQ ciliary localisation, consistent with their physical association as a functional module, as in motile cilia. Experiments are underway to test putative null mutants for behavioural changes consistent with impairment in OLQ cilium mechanosensation.

In all, our study of the cilium motility components present in *C. elegans* aims to unveil a functional module within the OLQ mechanosensory cilia. Our unexpected results provide insights into a previously unknown mechanosensory mechanism for cilia. Furthermore, our discovery of motility proteins in a non-motile, sensory cilium suggests the possible existence of what we refer to as 'hybrid' cilia in other metazoans, including vertebrates/mammals.

511B **Solving a genetic paradox: how a tRNA synthetase became a killer** Polina Tikanova[1,2], Daniel Krogull[1,2], Julian Ross[1,2], Valeria Stefania[1,3], Andreas Hagmüller[1], Pinelopi Pliota[1], Jacqueline Okweri[1], Gang Dong[4], Eyal Ben-David[5], Alejandro Burga[11]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), [2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, [3]Evotec Gene Therapy, [4]Max Perutz Labs, Medical University of Vienna, Vienna BioCenter (VBC), [5]Illumina Artificial Intelligence Laboratory, Illumina Inc

Toxin-antidote elements (TAs) are selfish genes that spread in populations by poisoning non-carrier individuals. As their name suggests, TAs typically comprise two genes: a toxin and its cognate antidote. Remarkably, the selfish behavior of TAs only emerges when the two genes are in tight genetic linkage—located within a few kilobases from each other. From the evolution of their components to their extreme genetic linkage, the evolution of TAs remains shrouded in mystery. Here we shed light into their paradoxical inception by reporting the discovery and characterization of two novel TAs in the nematode *Caenorhabditis tropicalis* and showing that genetic linkage between their two components predates the evolution of their selfish behavior.

While performing crosses between two wild *C. tropicalis* isolates, NIC203 and EG6180, we identified a novel TA on the left arm of Chr. V. The toxin, *klmt-1*, evolved via gene duplication from *fars-3*, a highly conserved and essential gene encoding subunit of the Phenylalanine tRNA-ligase complex. The antidote, KSS-1, belongs to a family of poorly characterized nematode proteins. An Alphafold2 model revealed that the N-terminal region of KSS-1 is virtually identical to the F-box domain of human SKP2 despite sharing no homology. F-box proteins are involved in SCF complex-mediated ubiquitination and subsequent proteasomal degradation. We found that KSS-1 physically interacts with two SKP1 homologs using a yeast-two-hybrid binding assay. Furthermore, KSS-1 directly binds KLMT-1 *in vitro*. Overall, our results suggest that the antidote directly binds the toxin and targets it for degradation via the SCF complex. Furthermore, we discovered and mapped a second TA on Chr. II. The toxin, *pzl-1*, also evolved from *fars-3* and its antidote, *kss-2*, is 64,6% identical to *kss-1*, but the antidotes are highly specific to their toxins.

These findings prompted us to examine the original *fars-3* locus more closely. Unexpectedly, we identified two *kss-1* paralogues immediately upstream of *fars-3*, which we hypothesize regulate FARS-3 turnover and maintain the stoichiometry of the tRNA-ligase complex. Thus, the genes that gave rise to both TA pairs were in genetic linkage prior to evolution of their selfish behavior. Our findings suggest that gene pairs consisting of a degrader and its target are the perfect substrate for the evolution of TAs. This is because if the target becomes toxic, the antidote is already in place, solving the longstanding "the chicken or the egg" paradox.

512B **A novel selfish gene that selectively exploits mitochondria to subvert Mendelian segregation** Alevtina Koreshova[1,2], Andreas Hagmüller[1], Alejandro Burga[1]1Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), [2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna

Mitochondria are essential for energy production, but also play a critical role in evolution. High mutation rates in mitochondrial DNA can cause genetic incompatibilities between nuclear- and mitochondria-encoded genes, preventing gene flow between populations and ultimately, leading to speciation. However, whether mitochondria play additional roles in shaping populations is largely unexplored. Here we report a new form of mitonuclear incompatibility that is caused not by the lack of compensatory mutations in co-evolving nuclear- and mitochondria-encoded genes but by a novel Toxin-Antidote element (TA) exploiting mitochondria for its own survival.

TAs are selfish genes that spread in populations by poisoning individuals that did not inherit them from their parents. While performing crosses between two natural isolates of the nematode *Caenorhabditis tropicalis*, we found seven maternal TAs. One of them was unique for the toxicity being entirely dependent on the mother's mitochondrial genotype: only homozygous non-carrier embryos carrying TA's cognate mitochondria were affected. This strongly suggests that the target of the toxin is encoded by the mitochondria.

To uncover the molecular mechanism underlying this phenomenon, we used recombinant near-isogenic lines and CRISPR/Cas9 homology-directed repair to fine map and validate the genes coding for the toxin and the antidote. A toxin null allele is sufficient to abrogate the toxicity, while genomic knockout of both toxin and antidote rendered the line susceptible to the wild-type TA. Interestingly, overexpressing of the toxin under the control of the heat shock promoter phenocopies the toxicity observed in genetic crosses but only when induced prior to gastrulation—later embryonic or larval stages are not affected. This, together with the fact that the toxin is not damaging the mother's gonad, suggests that the toxin may require an activation step to become fully active. Given that the toxin fused with N-terminal FLAG tag appears to have higher molecular weight on a Western blot than the analogous toxin with C-terminal FLAG tag, we hypothesize that there is N-terminal cleavage being disrupted by the N-terminal FLAG tag. This cleavage may be responsible for the toxin activation in early embryos.

The antidote belongs to the family of F-box domain proteins, which typically targets proteins for degradation via proteasome-mediated ubiquitination, but lacks the N-terminal F-box domain itself, suggesting that it may compete for binding with the mitochondrial target. We are now assessing the subcellular localization, structure, and protein interactions of the toxin and antidote to unveil their mechanism of action. Understanding how the toxin kills mitochondria of a specific genotype may assist us in mitochondrial genome editing that currently lacks efficient tools for genotype selection.

513B **How nematodes acquired the specific relationship with their vector beetles?** Haru Kirino[1], Noritoshi Maehara[2], Ryoji Shinya[1]1Agriculture, Meiji University, [2]Forest Entomology, Forestry and Forest Products Research Institute

Dauer larva is a vital phoretic stage in the life cycle of nematodes because it is specialized for various stress resistance and long-term survival without feeding. In *Caenorhabditis elegans,* dauer formation is promoted when the amount of ascarosides, the index of population density, increases. However, the stimuli to induce dauer formation may be different in the other nematode species. For example, dauer larvae of *Bursaphelenchus xylophilus,* the causal pathogen of pine wilt disease, appear only in the presence of the specific vector cerambycid beetle *Monochamus alternatus*. In this study, we focused on the mechanisms of dauer formation in *B. okinawaensis*, a close relative of *B. xylophilus*. Although *B. okinawaensis* is vectored by a specific *Monochamus* beetle*,* its dauer formation is readily induced even in the absence of its vector beetles, suggesting that *B. okinawaensis* undergoes an evolutionary process to develop a close relationship with the vector beetles. Here, we investigated the dauer-inducing conditions of *B. okinawaensis*, and compared them with those of *C. elegans* and *B. xylophilus* to reveal how nematodes acquired the specific relationship with their vector beetles. To determine the effect of population density on the dauer formation of *B. okinawaensis*, we propagated the nematodes with a crude extract of the cultured nematodes expected to contain dauer-inducing pheromones. The percentage of dauer larvae was significantly higher in the treatment group with the crude extract (25.7%; t-test, $p < 0.05$). In addition, the dauer formation of *B. okinawaensis* tended to be enhanced at higher temperatures with the crude extract as well as that of *C. elegans.* Furthermore, to clarify the effect of vector beetles on the dauer formation of *B. okinawaensis*, we propagated the nematodes with *M. alternatus* pupae until the beetles eclosed, and found that 22.8% of total nematodes developed into dauer larvae (t-test, $p < 0.01$). Therefore, *B. okinawaensis* dauer larvae can be induced by not only the density of nematodes but also the presence of the vector beetles. However, only 1.3% of dauer larvae were successfully transferred to *M. alternatus,* and this transfer rate is significantly lower than that of *B. xylophilus* to the beetles. These results suggest that the specific relationship with the vector beetles in *Bursaphelenchus* nematodes evolved first through inducing dauer formation by vector-specific signals, followed by acquiring the ability to transfer to the vectors.

514B **The *C. elegans* microbiome buffers the effects of pathogenic *Stenotrophomonas* bacteria on host health and fitness** Ashley E. Foltz, Michael A. Herman School of Biological Sciences, University of Nebraska - Lincoln

Bacteria are the sole food source of *C. elegans* and while many must be advantageous to their health, some are detrimental. *Stenotrophomonas* bacteria are abundant members of the natural *C. elegans* core microbiome described by Zhang *et al*. (2017). Surprisingly, we and others have shown that many *Stenotrophomonas* isolates are detrimental to *C. elegans* health (Samuel *et al*. 2016, White *et al*., 2016, Radeke and Herman, 2020). This has led us to ask several questions about the interactions that individual microbiome members have with other microbes, with the host, and how the entire microbiome community interacts with the host. We are characterizing the role of *Stenotrophomonas* in the microbial community to better understand these interactions. Our approach is to use an experimental microbiome (CeMbio) (Dirksen *et al*., 2020) to determine the effects of perturbing microbiome composition by substituting various *Stenotrophomonas* strains into the community. We have found that individual CeMbio members have varying effects on host health, indicated by survivorship, compared to the whole community. We used the effects of individual *Stenotrophomonas* strains on *C. elegans* health in monoculture and their isolation source to select strains for substitution experiments. We found that *C. elegans* survivorship is not significantly affected by most of the perturbations, although communities with more pathogenic *Stenotrophomonas* strains resulted in reduced survivorship. This suggests that the CeMbio community buffers the detrimental of effects of *Stenotrophomonas* on host survival. We also found that fecundity and development time, used as measures of host fitness, show similar results as the survivorship experiments. Interestingly, the effects of *Stenotrophomonas* on *C. elegans* in monoculture as compared to the effects of their respective substituted microbiomes suggests there may be a tradeoff. Specifically, the community appears to buffer the effects of highly pathogenic *Stenotrophomonas* strains, but also limits the beneficial effects of non-detrimental *Stenotrophomonas* strains. To better understand this tradeoff and buffering effect, we have investigated the community composition of the substituted communities. Preliminary results indicate that a lower relative abundance of detrimental *Stenotrophomonas* strains might contribute to the buffering effects observed in the community setting.

515B **Halophile nematodes live in America's Dead Sea** Julie Jung[1], Tobias Loschko[2], Shelley Reich[1], Michael Werner[11]University of Utah, [2]Max Planck Institute for Biology

Extremophiles can reveal the origins of life on Earth and the possibility of life elsewhere. Yet, most identified extremophiles are single-cell microbes, leaving gaps in our knowledge concerning the origins, or habitable limits, of multicellular organisms. Here, we report the recovery of roundworms (nematodes) from the Great Salt Lake, UT, a terminal lake referred to as "America's Dead Sea" due to its extreme salinity. Phylogenetic divergence and comparison to sampling efforts from Owens Lake, an analogous terminal saline lake in the Great Basin, suggest that they represent multiple previously undescribed species of Monhysteridae, the dominant nematode family in the abyssal zone and deep-sea hydrothermal vents. Nematodes in the GSL are specifically enriched in microbialites – organosedimentary structures which were once abundant on early Earth. Nematode:bacteria associations within microbialites hint at convergent mechanisms of survival and adaptation, and may reflect ancient animal:microbe interactions.

516C **Conjugative plasmids are necessary for adherence of commensal-like bacteria to *C. elegans* intestinal epithelium** Dalaena E Rivera[1], Gregory Jordan[1], Emily Morgan[1], Kayla Poirier[1], Nathan Habte[1], Marie-Anne Félix[2], Robert Luallen[11]Biology, San Diego State University, [2]Institut de Biologie, École Normale Supérieure

The persistence of conjugative plasmids in bacterial populations allow for bacteria to adapt to a wide array of environments, including host-associated niches. We have discovered that a *C. elegans* microbiome bacterium, LUAb3, contains a novel, conjugative plasmid required for colonization of the gut lumen. LUAb3 was originally found through ecological sampling of wild *Caenorhabditis* nematodes. Through light and fluorescent microscopy, we observed LUAb3 directionally binds to the *C. elegans* intestinal epithelium. Lifespan and brood size assays of LUAb3 colonized *C. elegans* showed the bacteria has a neutral effect on host fitness, suggesting it is best classified as commensal-like. Additionally, we found that LUAb3 actively proliferates and is maintained in the lumen of the intestines throughout the lifespan of the worm, colonizing nearly 90% of the anterior-posterior length of the worm in ~98% of the worm population. Whole genome sequencing and ANI analysis identified LUAb3 as Gram-negative bacterium, *Lelliottia jeotgali*. When we mapped the LUAb3 genome to the complete published genome of the type strain of *L. jeotgali*, we found that LUAb3 contains a novel, conjugative plasmid (pLUAb3-52k) encoding for a Type IV secretion system (T4SS) and a type IV pilus.

To determine the role of this plasmid in bacterial adherence to the *C. elegans* intestinal epithelium, we cured LUAb3 of pLUAb3-52k and found complete loss of adherence in *C. elegans*, suggesting this plasmid is necessary for adherence. We isolated three additional *Lelliottia* strains from wild *Caenorhabditis* isolates from around the world and found that each can adhere in the *C. elegans* intestine similarly to LUAb3. Interestingly, we found that each of the *Lelliottia* isolates has a large plasmid divergent from pLUAb3-52k, but contains highly conserved operons encoding for a T4SS and a type IV pilus. 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. For example, the plasmid in LUAb3 is more phylogenetically related to a plasmid in a strain of *Enterobacter asburiae* isolated from baboon feces than to plasmids isolated from *Lelliottia* spp. in *C. elegans*. Altogether, the data suggest 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.

In conclusion, we have discovered that a *C. elegans* microbiome bacterium requires a conjugative plasmid for colonization of the gut lumen and have identified related plasmids in several colonizing *Lelliottia* strains. We aim to understand the role of these plasmids in the capacity of commensal bacteria to adhere and form niches in the intestine and whether host binding is transferrable to non-adhering bacteria via conjugation.

517C **Gene family evolution of Argonaute regulatory proteins across the *Caenorhabditis* phylogeny** Daniel D Fusca, Vicky Zhu, Katja R Kasimatis, Asher D CutterDepartment of Ecology & Evolutionary Biology, University of Toronto

Despite their extremely conserved outer morphologies, most *Caenorhabditis* species are highly divergent from each other at the genomic level. Genetic divergence between species could be caused by adaptive evolution driving the fixation of beneficial genetic variants, but the extent to which adaptation contributes to genomic differences between *Caenorhabditis* species remains poorly understood. One potential target for adaptation in *Caenorhabditis* are Argonaute proteins, a group of regulatory proteins that alter the expression of target genes and/or repetitive DNA by binding to small noncoding RNAs. Some small RNA pathways evolve rapidly in nematodes, including large gene family expansions that have created many nematode-specific Argonautes, raising the possibility that Argonautes may be evolving adaptively in *Caenorhabditis*. To characterize the evolution of Argonaute regulatory proteins across a diverse range of *Caenorhabditis* species, we used available reference genomes and transcriptomes to identify and annotate Argonaute genes in 51 *Caenorhabditis* species. In total, we identified 1214 genes belonging to 11 gene families, which represents the most comprehensive set of *Caenorhabditis* Argonautes to date. Cataloguing the Argonautes found in these 51 species revealed that gene family size varied among Argonaute families and among species. Some families have highly conserved copy numbers (e.g., ALG-5 is present in exactly 1 copy in 50 species), whereas others vary substantially (e.g., ERGO-1 was not detected at all in 8 species, but present in 5 or more copies in 9 other species). In addition to the apparent complete loss of gene families in some species, we also discovered putative Argonautes that bear little sequence similarity to any known *Caenorhabditis* Argonaute, representing potential novel Argonaute families. To complement our analyses of gene copy number evolution, we have also begun to analyze rates of sequence evolution. Comparing evolutionary rates between species isolated from tropical vs temperate habitats, we found that the Argonaute CSR-1 evolved faster in tropical species, consistent with the possibility of relaxed purifying selection in tropical species. Our findings document the evolution of substantial Argonaute diversity in *Caenorhabditis*, potentially contributing to differences between species in post-transcriptional gene regulatory controls, genome size and content, and adaptive evolution.

518C **Are *C. elegans* behaviors relevant for the worm in the real world? Studying worm behavior in its native ecology** Jongmin Park[1], Rocel Amor Indong[1], Jin I Lee[1], Jin-Kyung Hong[2], Eun Sun Lyou[2], Tae Kwon Lee[21]Biological Science and Tech-

nology, Yonsei University Mirae Campus, [2]Environmental and Energy Engineering, Yonsei University Mirae Campus

Behaviors are an adaptive product of the ecology and environment that the animal is exposed to, increasing its reproductive fitness over many generations. The nematode *Caenorhabditis elegans* has been an excellent model species to study the molecules and circuitry that control behaviors such as response to environmental cues in the laboratory. However, normal laboratory culture is quite different from the native ecology of the worm. Large populations of *C. elegans* can be found thriving in rotting fruit and adjoining soil (Samuel et al, 2016). To examine the intersection between *C. elegans* ecology and sensory behaviors, we designed a soil-fruit natural habitat (soil-fruit habitat) in the laboratory that simulates nematode true ecology. Apples were placed atop soil and exposed to day/night, temperature and humidity cycling. After a period of rotting, 30 L1 larvae were added to the habitat and the growing worm population was tracked inside layers of the apple and layers of the soil. We observed that *C. elegans* can grow and reproduce more or less at a rate similar to normal laboratory culture in the soil-fruit habitat. We also found that *C. elegans* migrates between shallow and deep layers of both soil and fruit within this habitat. This movement of *C. elegans* appears to depend on the time of cultivation and the developmental stage of the worms. By analyzing microbial populations in each of the areas over time, we plan to examine whether migrations of the worms are correlated with qualitative and quantitative markers of the microbial communities. Finally, we plan to study whether reproductive success and/or migration in the soil-fruit habitat depends on specific behaviors (ie. chemotaxis or avoidance, thermotaxis, foraging strategies, etc.) by placing mutants of sensory behaviors in the habitat and tracking reproductive success and migration. Our study reveals a foundation of further exploration of *C. elegans* behavior based on natural ecological parameters, and this information can lead to a well-established natural-based research approach for *C. elegans* study in the future.

519C **Quantitative high throughput measurement of selection in an animal system via novel barcode library transgenesis** Zachary C Stevenson, Ellie A Laufer, Patrick C Phillips Biology, University of Oregon, Institute of Ecology and Evolution

Caenorhabditis elegans is a widely used model organism for studying various biological processes, including neurobiology, aging and experimental evolution. C. elegans have many advantages for experimental evolution, such as a rapid life cycle, large brood size, the capacity to freeze and revive populations, self-fertilization reproduction and easy genetic manipulation via CRISPR. We used C. elegans as the first genomically-barcoded experimental evolutionary animal model to compete two different strains under various concentrations of ivermectin as a selective pressure. We used N2 as our susceptible strain and JD608 as our resistance strain. We introduced a genomic barcode sequence into lineages of each strain using CRISPR genome editing by TARDIS (Transgenic Arrays Resulting in Diversity of Integrated Sequences), a high-throughput library transgenesis method that allows inducible integration of individual sequences from transgenic arrays into engineered genomic sites. Mixed populations are grown in liquid medium for approximately five generations with various concentrations of ivermectin. By adopting a liquid protocol, we can grow populations in the millions, making this one of the largest animal experimental evolutions to date. We transferred 10% of the mixed population to fresh medium every five days for a total of five transfers. We then quantified the relative frequency of each strain in the mixed population by PCR amplification and sequencing of the barcode region. Barcode frequencies are then used to measure the fitness of the individual lineages in the population. We found that at low concentration of ivermectin, the sensitive strain holds an advantage, while higher concentrations tend to favor the resistant strain. We also find that the sensitive strain tends to develop slower on increasing ivermectin concentrations, whereas the resistant strain remains mostly stable. We hypothesize this time to reproductive adult is the leading cause of the advantage for either genotype in their respective advantaged conditions. Our results demonstrate that C. elegans can be used as a high-throughput barcoded animal experimental evolutionary model to compete different strains and provide replicated high-resolution estimates of fitness.

520C **Genetic assimilation underlies the emergence of novel neuronal functions.** Andrea Millán Trejo[1], Carlos Mora[2], Adrián Tarazona[1], Rafael Alís[1], Carla Lloret-Fernández[3], Miren Maicas[1], Arantza Barrios[3], Nuria Flames[11]Developmental Neurobiology, Instituto Biomedicina Valencia-CSIC, [2]Developmental Neurobiology, Instituto Biomedicina Valencia - CSIC, [3]Cell and Developmental Biology, University College London

One unsolved question in Evolutionary Biology is how simple nervous systems modify their neuron types to acquire new functions and to increase neuronal diversity. In this study we investigate changes in gene regulatory networks that underlie the emergence of new neuronal identities in the *Caenorhabditis* genus.

All species in the *Caenorhabditis* genus share an invariable cellular lineage that generates 302 neurons. We find VC4 and VC5 neurons, which are cholinergic in *C. elegans* strongly stain for serotonin in *Angaria* subgroup species (including *C. angaria* and four additional *Caenorhabditis* species of the *Angaria* subgroup).

Gained expression of the serotonin re-uptaker (SERT/*mod-5)* in *C. angaria* VC4 and VC5 allows these neurons to re-uptake serotonin from the neighbouring HSN serotonergic neuron. Expression of SERT/*mod-5* in *C. elegans* VC4 and VC5 is sufficient to provide the serotonergic phenotype while *C. angaria* SERT/*mod-5* mutants lack VC4 and VC5 serotonergic staining.

Our gene regulatory studies show that a pre-existing SERT/*mod-5* enhancer, active in NSM and ADF serotonergic neurons in other Caenorhabditis species including *C. elegans*, has been co-opted to be active in VC4 and VC5 neurons of the *Angaria* species. We determine that *C. angaria* SERT/*mod-5* enhancer has been recruited as a new direct target of UNC-4, a homeodomain transcription factor that acts as terminal selector in *C. elegans* VC4 and VC5 neurons. Importantly, UNC-4 activates *C. angaria* enhancer but not *C. elegans* SERT/*mod-5* in VC4 and VC5 neurons.

Next, we hypothesized that genetic assimilation, a process where a phenotype, initially responsive to the environment becomes fixed in a specific state, could underlie the *C. angaria* VC4 and VC5 serotonergic phenotype. Indeed, analysis of *Caenorhabditis* wild isolates reveals plasticity in VC4 and VC5 serotonergic staining, as Hawaiian CB4856 isolate shows moderate but significant levels of VC4 and VC5 serotonergic staining. We find that in *C. elegans* low UNC-4 levels together with chromatin repression through H3K9 methylation ensures silencing of endogenous SERT/*mod-5* enhancer to impede VC4 and VC5 serotonergic phenotype. Finally, we find that the VC4 and VC5 serotonergic phenotype impacts on egg laying behaviour.

In summary, our study provides new insights into the regulatory mechanisms underlying neuron type diversification and supports genetic assimilation as a mechanism for evolutionary novelties.

521C **_C. elegans_ as a model to investigate the interactions of soil invertebrates with microplastics** David J Elliott[1], Rachel J McMullan[1], Simon Collinson[1], Carl J Boardman[21]Life, Health and Chemical Sciences, The Open University, [2]School of Engineering and Innovation, The Open University

Microplastics are a ubiquitous environmental contaminant. The potential biological consequences of microplastics to terrestrial fauna include skin damage and oxidative stress in earthworms, neurodegeneration, reduced lifespan, altered metabolism, oxidative stress and brood size changes in nematodes, and metabolic disturbance and accumulation of microplastics in the internal organs of mice. Additional risk is posed by trophic transfer, where microplastics ingested by soil invertebrates accumulate at higher trophic levels via predation, potentially magnifying the effects in these animals. Together, these consequences may pose risks to the greater health and functioning of terrestrial ecosystems.

Caenorhabditis elegans has been used to elucidate the potential biological harms of microplastic ingestion, but their behavioural interaction with microplastics *in natura* is unknown. The majority of microplastic exposure experiments are conducted using readily ingestible, uniform size, pristine spherical microplastics under conditions eliminating food choice and free behaviour. Here, we use *C. elegans* exposed to microplastic particles of polyethylene terephthalate (PET) and poly lactic acid (PLA) with irregular morphologies and in a range of size from 0 to 38 µm as a model to investigate how soil invertebrates interact with environmentally representative microplastics when allowed to freely behave. *C. elegans* were exposed to different concentrations of these microplastics in the presence of a range of OP50 concentrations and free association with contaminated food patches and the rate of ingestion were measured. A strong relationship between microplastic concentration and food availability with respect to particulate ingestion was found, with the presence of food significantly reducing the risk of ingestion. Retention of microplastic particles in the digestive tract of *C. elegans* is dependent on food availability and active feeding behaviour. Additionally, spatial and social feeding behaviour was significantly affected by microplastic concentration, with animals actively avoiding high concentrations. The mechanisms governing this behaviour are being elucidated using a range of sensory neuron defective mutants. This work will contribute to a better understanding of how microplastic contamination may affect terrestrial invertebrates and the wider ecosystems they inhabit.

522C **Evolution of genes that negatively regulate cell proliferation in nematodes** NIKITA JHAVERI[1], Helen M Chamberlin[2], Bhagwati P Gupta[11]McMaster University, [2]Molecular Genetics, Ohio State University

The nematode vulva is an established organ for comparative studies due to its invariant cell lineages and well-defined cell fates. We are using *C. elegans* and *C. briggsae* to understand evolutionary changes in the mechanism of vulval development, specifically the synthetic multivulva (synMuv) genes that regulate chromatin mediated gene expression. In *C. elegans*, animals carrying mutations in both class A and B synMuv genes exhibit excessive cell proliferation due to inappropriate induction of vulval precursor cells (VPCs). While synMuv genes have been studied extensively in *C. elegans*, nothing is known about their orthologs in other nematodes. We have generated CRISPR alleles of three different synMuv orthologs (*lin-35*, *lin-8*, and *lin-38*) in *C. briggsae* and found that while *lin-35;lin-8* and *lin-35;lin-38* double mutants in *C. elegans* are 100% Muv, the corresponding doubles in *C. briggsae* display no such synthetic interaction. Our data has also revealed differences in other phenotypes of *lin-35* mutants, namely transgene silencing, high temperature larval arrest (HTA), and mis-expression of germline genes in the soma. Furthermore, phylogenetic analysis showed that class A synMuv genes are more diverged in *Caenorhabditis* nematodes compared to those belonging to class B. Together with the previous work from our lab on *ivp* (Inappropriate Vulval cell Proliferation) class of genes, these findings demonstrate significant changes in the mechanism of vulval development in *Caenorhabditis* nematodes. We are currently carrying out transcriptomic profiling in *lin-35* and *ivp* mutants to understand changes in signaling pathways and genetic

networks. Overall, our work has uncovered that despite a conserved morphology of the vulva in *C. elegans* and *C. briggsae*, there are substantial evolutionary changes in underlying genetic networks.

523C **A tale of two nematodes: Evolution of neuronal fate specification in *C. elegans* and *P. pacificus*** Yasmin H. Ramadan[1], Steven J. Cook[1], Curtis Loer[2], Hanh Witte[3], Ralf Sommer[3], Oliver Hobert[11]Biological Sciences, Columbia University, [2]Biology, University of San Diego, [3]Max Planck Institute

Although we have made progress in identifying gross differences between nervous system function and organization across species, what remains to be discovered is whether there are conserved molecular factors that drive these evolutionary changes. We aim to answer this question by comparing cell fate specification in the nervous systems of two distantly related nematode species, *Caenorhabditis elegans* (*Cel*) and *Pristionchus pacificus (Ppa)*. Using CRISPR, we have engineered null mutations in a series of transcription factors that have been well-characterized as terminal selectors, or master regulators of neuronal fate in *Cel*. These include *unc-42*, *unc-3*, *unc-4*, *unc-30*, *vab-7*, *mec-3*, and *unc-86*, which have all been shown to regulate the identity of neurons important for locomotion and sensation in *Cel*. Most of the *Ppa* orthologs of these genes show conservation of their roles in nematode behavior, namely locomotion and sensation of mechanosensory or nociceptive cues. (Some of these genes, for which we have epitope-tagged strains, also show identical or similar expression patterns.) Others, like *unc-30*, show divergence in their terminal selector roles: in *Ppa-unc-30*, GABA identity in the Ventral Nerve Cord (VNC) is retained, rather than lost, and mutant animals do not "shrink", which is expected when GABA is absent from the VNC. Together, these comparative analyses allow us to not only characterize neuronal fate specification in a new model organism, but also investigate how this specification is conserved across species in some areas of the nervous system while in others it may have diverged.

524C **Evolution of fem-1 activity in Caenorhabditis** James F Kennedy, Ronald E EllisMolecular Biology, Rowan GSBS

Hermaphrodite sex determination is shared between the *Caenorhabditis* species *C. elegans*, *C. briggsae*, and *C. tropicalis*, but this evolution of this trait is thought to have occurred independently based on phylogeny. The core pathway that makes this determination shares large similarity in the *Caenorhabditis* genus among which are the FEM complex proteins: FEM-1, FEM-2, and FEM-3. These proteins were discovered in *C. elegans* where corresponding genes are necessary for male sex determination as evidenced by null mutants producing feminizing mutations in the soma and germline in both *XX* and *XO* animals. This phenotype is not shared in *C. briggsae* where *fem-2* and *fem-3* null mutants in both *XX* and *XO* animals are hermaphrodites. This difference suggests a divergence in the role of the FEM complex in these two species.

This comparison is accentuated by the isolation of *C. tropicalis fem-1(v426ts)* by genetic screening and its failure to compliment the null allele *fem-1(v466)* created by CRISPR/Cas9. Mutants with these *fem-1* genes closely resemble what we know in corresponding *C. elegans* mutants. Both *XX* and *XO* animals develop as females, and there is a strong maternal rescue in null mutants. My research began with developing a *fem-1* null mutant by CRISPR/Cas9 injection in *C. briggsae*.

The mutant strain of *fem-1(v508)* showed an identical phenotype to *C. briggsae fem-2* and *fem-3* null mutants producing fertil e *XX* and *XO* hermaphrodites. *XO* hermaphrodites were verified through RT-PCR of *her-1* mRNA and *unc* mutant genetic crosses. To place *fem-1* in the sex determination pathway, double mutants of this gene with its direct upstream (*tra-2*) and downstream (*tra-1*) genes were produced. The expected results were received with *tra-1(v181); fem-1(v508)* mutants being *XX* males and *tra-2(nm1); fem-1(v508)* mutants being *XO* hermaphrodites. While the FEM proteins were expected to act as a complex, a null triple mutant of *fem-2(nm27)*; *fem-3(nm63) fem-1(v517)* was produced by CRISPR/Cas9 of *fem-1* in an existing *fem-2; fem-3* double mutant. This mutant showed an identical phenotype to individual mutants in the *fem* genes.

A complex relationship between the FEM proteins and germline development is suggested by oogenesis in *tra-1; fem* double mutants in *C. elegans*. An attempt to replicate this was performed in *C. briggsae* by germline scoring of *tra-1(v181)* mutants with corresponding *fem* genes. A spermatogenesis then oogenesis switch was seen in these *tra*-1 males. This result suggest that the *fem* genes serve an unknown downstream function in both *C. elegans* and *C. briggsae*.

525C **Measuring the fitness of a large panel of wild *C. elegans* isolates against pathogenic bacteria** Yin Chen Wan[1], Meng Xiao[1], Calvin Mok[2], Aaron Reinke[11]Molecular Genetics, University of Toronto, [2]University of Toronto

Given the threat of infectious disease to human health, there is growing interest in understanding how pathogens interact with humans. Observations of natural variation to disease susceptibility, combined with the reported conserved mechanisms of infection between *Caenorhabditis elegans* and mammals, suggest that wild strains are a useful resource of variation to dissect host response to infection. A multiplexed sequencing-based screen, PhenoMIP, was used to measure phenotypes in 22 wild *C. elegans* strains against P *Pseudomonas aeruginosa* (PA14) and *Pseudomonas vranovensis*. From this assay, we identified several strains which are sensitive or resistant to Pseudomonas aeruginosa or Pseudomonas vranovensis. Our current goal is to expand the PhenoMIP assay to examine a greater number of wild isolates, to identify additional strains with variations in fitness to patho-

gens such as bacteria and microsporidia. Overall, the goal of this work is to identify key molecular mechanisms in host-pathogen interactions between *C. elegans* and bacteria that serves as a model to understand these types of infections in humans.

526C **Understanding neuron type evolution at single-cell resolution** Adrián Tarazona Sánchez, Antonio Jordán-Pla, Nuria Flames Bonilla Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia

How gene regulatory networks change in evolution to give rise to complex nervous systems with thousands of different neuron types is a key unsolved question in Evolutionary Biology. *C. elegans* nervous system is well-characterized, with available transcriptome for the 118 different neuron types in the hermaphrodite. However, little is known about the gene expression profiles of the neurons in other *Caenorhabditis* species. In this study, we propose the analysis of other *Caenorhabditis* species, specifically *C. briggsae*, to provide valuable insights into how neurons could acquire new functions and evolve into new cell types.

In this project, we will use single cell technologies to obtain the transcriptome and chromatin accessibility profile of *C. briggsae*. We have already obtained RNAseq data for 7348 prospective neurons in *C. brigssae* and identified 21 different neuron types. We are now integrating this data with our own and published *C. elegans* datasets to identify changes in gene expression. This comparison is a crucial step towards understanding the principles that govern the development and evolution of neurons across species. We envision that our work will increase our understanding on the molecular mechanisms that drive the evolution of complex nervous systems and provide a framework for future studies in this area.

527C **Molecular changes of a chemosensory component in males may facilitate the mating system transition from dioecy to androdioecy in *Caenorhabditis* species** Harini Kannan, King L ChowLife Science, The Hong Kong University of Science and Technology (HKUST)

Some *Caenorhabditis* species evolved to acquire an androdioecious status from an ancestral dioecious state. In the process, females of ancestral species have accumulated genetic mutations leading to events such as self-sperm production, self-sperm activation and reduction of sex pheromone production. Despite the dramatic reduction of sex pheromone produced by *C. elegans* hermaphrodites, modern day *C. elegans* males have retained their ability to respond to sex pheromones. We show that *C. elegans* males adopt a common chemosensory transduction mechanism shared for processing different volatile odors, e.g., diacetyl and pyrazine. While androdioecious hermaphrodites have attenuated production of volatile sex pheromones and are unlikely causing sex pheromone saturation in the environment, androdioecious males exhibit a robust pheromone-mediated olfactory habituation with high specificity. This feature is a distinct trait that could have facilitated androdioecy establishment. We show that mating cue-associated response of androdioecious *Caenorhabditis* males could be reversed to follow the behavioral pattern of dioecious *Caenorhabditis* males by molecular replacement of genetic components. We further identified critical protein domain that confers such specificity. Based on these findings, we postulate that dioecious males have accumulated genetic variations largely impacting chemosensation towards sex pheromones and changes that lead to a perception of population decline. Both traits have provided conducive conditions leading to establishment of androdioecy and self-fertilization. [The project is supported by Research Grants Council, Hong Kong]

528C **Functional analysis of Hox genes in the nematode *Panagrolaimus* sp. PS1159** Viktoria Hellekes, Denise Claus, Maja Jarzabek, Michael Kroiher, Philipp Schiffer Institute for Zoology, University of Cologne

Hox genes encode highly conserved transcription factors involved in patterning the anterior-posterior (AP) body axis in embryonic development of many bilaterian animals. Canonically, 8 Hox genes can be found in a cluster organized in four groups - anterior, group 3, central, and posterior. In many cases expression of the genes along the animals AP axis correlates with their genomic location (collinearity). In the nematode model *C. elegans* however, only 6 Hox genes in 3 groups are present - the anterior *ceh-13*, the central *lin-39* and *mab-5*, and posterior *egl-5*, *nob-1* and *php-3*. The collinearity-rule is broken, as *ceh-13* lies downstream of the middle Hox gene *lin-39*. Finally, only three genes are essential during embryogenesis. The LIN-39 protein plays an important role in vulva development, a greatly studied process in *C. elegans*.

Panagrolaimus sp. PS1159 is a free-living triploid nematode reproducing parthenogenetically. We are utilizing the species to comparatively study development, evolution, and alternative reproduction strategies in nematodes that are only remotely related to the model system. We aim to study and functionally analyze Hox genes in PS1159 using the CRISPR/Cas9 approach. Here, we describe the successful use of the system to generate mutations by microinjection of CRISPR components targeting the orthologue of *lin-39* into the germ line of PS1159. We found progeny with deformed vulva phenotypes, genotyped the region spanning the target site and found that in a vulvaless mutant all three alleles were affected. In specimens with weaker phenotypes either 1 or 2 alleles were mutated. Surprisingly, in the established mutant lines, we find the rare occurrence of worms with *Panagrolaimus* male traits. These include spicules in the tail region or intersex phenotypes with both, vulva, and remains of spicules. So far, we have not observed any mating behavior, as well as the presence of active sperm is still unknown,

suggesting these represent nonfunctional “pseudo-males”. We are now in the process of identifying targets for *mab-5* and aim to analyze hox genes not only in PS1159, but a broad range of nematodes in the phylum.

529C **Gut microbes and host interaction determine the lifespan in *C. elegans*** Xusheng Hao, Limeng Liu, Yongqing Guo, Ye Tian Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

The gut microbiome has increasingly been recognized as an important contributing factor in the health and longevity of its host, yet the complexity of the microbiota and the diversity of the host’s genetic background have made it difficult to explore the effects of gut bacteria on the longevity of individuals with genetic differences. To better understand the impact of gut bacteria on health and longevity, we conducted a lifespan screening of 137 isolates of *Arabidopsis* root-derived bacterial collections. Our results revealed 9 genera of bacteria isolates that significantly extended the lifespan of wild-type *Caenorhabditis elegans*. Surprisingly, *Variovorax sp.* Root473 significantly increased the lifespan of wild-type N2 worms and improved age-related physiological deteriorations, yet remarkably shortened the lifespan of *skn-1(zj15)* mutants lacking the ability to activate the oxidative stress response. Furthermore, dietary supplementation with the antioxidant N-acetylcysteine significantly reversed the shortened lifespan of *skn-1* mutants fed on Root473, indicating that genetic variations in host oxidative stress capacity may determine whether Root473 is beneficial or detrimental to *C. elegans* lifespan. Transcriptome analyses revealed that the ferroptosis pathway was overactivated in *skn-1* mutants grown on Root473, and supplementation with the iron chelator 1,10-Phenanthroline strongly reversed the shortened lifespan of *skn-1* worms fed on Root473. Next, we observed that *skn-1* worms with Root473 exhibited severe damage to membrane integrity, especially in the form of vulva protrusion, resulting in an accelerated aging phenotype. In contrast, mutants without vulval formation recovered the lifespan extension in *skn-1* worms with Root473. In conclusion, our results indicate that the microbiome and host interactions play a pivotal role in determining longevity, thus emphasizing the need for personalized microbial intervention to promote health and longevity.

530C **Assessing the impact of environmental versus genetic variation on vulval precursor cell fates and their possible evolution under pleiotropy** Charlotte Bouleau[1], Marie Marcaillou[2], Joao Picao-Osorio[2], Marie-Anne Félix[2], Christian Braendle[11]Institut de Biologie Valrose (iBV) “Sciences Naturelles” Université Nice Sophia Antipolis Faculté des Sciences, [2]Institut de Biologie de l’ENS (IBENS), Département de biologie, École normale supérieure, CNRS, INSERM, Université PSL

While the *C. elegans* cell lineage is mostly fixed, variation occurs for some cells, and evolution occurs intra-specifically and at a larger scale between species. The vulval precursor cell fates have proven to constitute a great system to study the quantitative spectrum of variation when confronted to various perturbations. In *C. elegans*, the main variation concerns the most anterior of the six vulval precursor cells, P3.p, which can either divide or not divide and then fuse to the hyp7 epidermal syncytium. In the *Oscheius* genus, the vraiation occurs on P4.p and P8.p.

Here we ask 1) whether environmental variation act in the same directions of phenotypic space as random genetic variation; 2) whether the system may evolve under selection for a pleiotropic genetic variant.

Variation of environmental parameters could in principle affect the same biochemical reactions in the developmental system as random genetic variation. However, quantitative differences in the relative effect of a wide range of environments versus random mutation can be expected, which may matter for evolution in varying environments. Picao Osorio et al. (see abstract) assessed the mutational variance (Vm) upon random mutation in two wild isolates each of C*. elegans*, C*. briggsae*, *Oscheius tipulae*, *O. onirici,* the genetic variance (Vg) in natural populations of these four species, and the interspecific divergence using available species in each genus. Here we scored vulval precursor cell fates under 40 environments in the same eight *Caenorhabditis* and *Oscheius* parental strains in which we produced random mutation lines. Although the environmental (Ve) and genetic variance (Vm and Vg) datasets overall show the same trend, we detect some differences between them.

To address the possibility of pleiotropic selection on another trait, we gathered a library of CRISPR edits of natural variants that have been shown to affect different phenotypes (thanks to the community!). We plan to assay them for their possible effect on vulval precursor cell fates. We expect that some of them may affect P3.p fate.

531C **Exploring the evolution of pesticide resistance in real-time** Luna Qingyang Li[1], Anthony Flemming[2], Alison Woollard[11]University of Oxford, [2]Sygenta

Pesticide resistance is one of the chief reasons for failed vector and pest control. Such failures have grave consequences, such as lives lost to vector-borne diseases, or significant reduction in crop yield with consequent threats to local food security. In comparison to antibiotic resistance, pesticide resistance receives little attention, yet, it remains a paramount challenge for disease control and agriculture.

Pesticide resistance is deemed pre-adaptive, meaning that a low level of genetic resistance is presumed to exist in a naïve pop-

ulation due to standing variation. This suggests that in order to move closer to an 'evolution-proof' management strategy, we need a detailed understanding of the evolutionary dynamics of the resistance alleles involved. To this end, we are using *Caenorhabditis elegans* as a model organism to understand genetic resistance dynamics through experimental evolution.

Exploratory studies have shown that *C. elegans* is a suitable model for understanding pesticidal modes of action, and moreover that this experimental system captures single-compound resistance dynamics well. Currently, the focus is on understanding pairwise-compound interactions, the associated resistance dynamics and evolutionary trade-offs. In parallel, we are developing high-throughput worm fitness assays based on food consumption, as well as quantitative genomics methods to probe the genetic makeup of a worm population. We hope to leverage antagonistic and synergistic pesticidal interactions to design improved resistance management strategies. Information derived from the experimental system will be integrated into theoretical models to generate broad insights on resistance evolution as well as strategies to better manage it.

532C **Higher-order epistasis shapes natural variation in germ stem cell niche activity** Sarah Fausett[1], Asma Sandjak[2], Bénédicte Billard[2], Christian Braendle[21]Biology and Marine Biology, University of North Carolina Wilmington, [2]institut de Biologie Valrose, Université Côte d'Azur, CNRS, Inserm

Natural quantitative variation in developmental processes must be driven by allelic variation. Yet, the genotype-phenotype relationships underlying developmental system variation are understudied due to their inherent complexity. Taking advantage of the simple *Caenorhabditis elegans* germline stem cell system, we characterized natural differences in the germ stem cell niche activity of two distinct wild isolates—measured as differences in germline progenitor zone (PZ) size. Through quantitative trait locus (QTL) analysis, we detected multiple candidate causal loci, including two large-effect QTL on chromosomes II and V. Resolving the chromosome V QTL, we show that the isolate with a smaller PZ exhibits a unique 148 bp deletion in the promoter region of the Notch ligand, *lag-2*, a central signal promoting germ stem cell fate and proliferation. As predicted, introducing this deletion into the isolate with a large PZ resulted in a smaller PZ. Unexpectedly, re-introducing the deleted ancestral sequence in the isolate with a smaller PZ further reduced PZ size. Using allelic replacement lines, we show that these contradictory phenotypic effects are due to epistatic interactions among the *lag-2* promoter, the chromosome II QTL, and additional loci in the genome. Although the *lag-2* deletion appeared to explain natural variation in germ stem cell niche activity, its effects across multiple genetic backgrounds were unpredictable due to higher-order epistasis. Studying the genetic architecture of quantitative developmental systems without taking into account its natural variation may be misleading, emphasizing the need for a better integration of developmental and quantitative genetics.

533C **A Mediator subunit imparts robustness to a polyphenism decision** Sofia Casasa, Eleni Katsougia, Erik J RagsdaleDepartment of Biology, Indiana University

Polyphenism is a type of developmental plasticity that translates continuous environmental variability into discontinuous phenotypes. Such discontinuity likely requires a switch between alternative gene-regulatory networks, a principle that has been borne out by mechanisms found to promote morph-specific gene expression. However, whether robustness is required to execute a polyphenism decision has awaited testing at the molecular level. Here, we used a nematode model for polyphenism, *Pristionchus pacificus*, to identify the molecular regulatory factors that ensure the development of alternative forms. This species has a dimorphism in its adult feeding structures, specifically teeth, which are a morphological novelty that allows predation on other nematodes. Through a forward genetic screen, we determined that a recently duplicated homolog of a Mediator subunit MDT-15/MED15, *P. pacificus* MDT-15.1, is necessary for the polyphenism and the robustness of the resulting phenotypes. This transcriptional coregulator, which has a conserved role in metabolic responses to nutritional stress, coordinates these processes with its effects on this diet-induced polyphenism. Moreover, this MED15 homolog genetically interacts with two nuclear receptors, NHR-1 and NHR-40, to achieve dimorphism: single and double mutants for these three factors result in morphologies that together produce a continuum of forms between the extremes of the polyphenism. In summary, we have identified a molecular regulator that confers discontinuity to a morphological polyphenism, while also identifying a new role for MED15 as a plasticity effector.

534V **Regulatory mode and genomic context determine natural gene expression variation in *C. elegans*** Avery Davis Bell, Han Ting Chou, Annalise B PaabySchool of Biological Sciences, Georgia Institute of Technology

To investigate the evolutionary and molecular underpinnings of gene expression variation in *C. elegans*, we performed RNA sequencing in the laboratory strain N2 and four wild strains with varying levels of genomic divergence from N2. Genotype strongly impacted gene expression: 34% of genes differed in expression across the five strains and 8% of these had zero expression one or more strains despite robust expression in at least one other strain. By examining DNA sequence coverage, we determined that >92% of differentially expressed genes likely had true RNA level differences, rather than appearing differentially expressed because of mapping bias.

We also examined global and allele-specific expression in the F1 offspring between N2 and each of the four wild strains, which allowed us to parse *cis*- versus *trans*-mediated effects. Of the many genes with *cis* regulatory changes, a large fraction had identical expression in the two parents, meaning they were buffered by compensatory *trans* changes. *Trans* changes often underpinned inter-strain differential expression.

Specific regulatory patterns might reflect trends in genomic location or nucleotide diversity. In fact, genes with strain-wise expression differences were enriched in variant-rich chromosome arms, and correspondingly harbored more nucleotide diversity than other genes. Conversely, genes with *cis* regulatory changes compensated in *trans* were enriched in chromosome centers and had lower nucleotide diversity. All F1s exhibited these patterns, regardless of the genomic divergence between their parents.

These observations hint 1) that gene expression may be under stabilizing selection as *trans* changes compensate *cis* changes and 2) that genomic context, *i.e.*, the recombination landscape of genetic variation, plays a large role in determining which genes are variably expressed (consistent with earlier eQTL studies) and in the modes of this variation. This study sheds light on the evolution of gene regulation and serves as a resource for the research community. Websites providing access to the data—including queries by gene for strain-wise differential expression, regulatory classification, and more—are available here: https://wildworm.biosci.gatech.edu/rnai/; https://wildworm.biosci.gatech.edu/ase/.

535V **The impact of the population size on the genome structure and evolution in *Caenorhabditis* nematodes** Anastasia A Teterina[1,2], John H Willis[1], Charles F Baer[3], Patrick C Phillips[11]Institute of Ecology and Evolution, University of Oregon, [2]Center of Parasitology, Institute of Ecology and Evolution, [3]Department of Biology, University of Florida

The impact of various evolutionary processes on the evolutionary trajectories of genomic components has long been a question of interest in genetics. *Caenorhabditis* nematodes of the 'Elegans' group are free-living species that have different reproduction modes (selfing and outcrossing) and thus different demographies and population structures. However, these species have a relatively conservative genomic organization. The selfing *C. elegans* has an estimated effective population size of about 10,000 individuals (Sivasunda et al. 2003, Barrière et al. 2005), whereas the outcrossing *C. remanei* has a population size one order of magnitude greater (Cutter et al. 2006, Teterina et al. 2023). *Caenorhabditis brenneri* is an outcrossing species (Rhabditida; Sudhaus & Kiontke 2007) known for its high genetic diversity. Nearly one in ten nucleotides in C. brenneri is polymorphic, making its population diversity level comparable to that of bacteria (Dey et al. 2013). This genetic diversity is presumably due to the vast population size of *C. brenneri*. In this study, we investigate the impact of the effective population size of species on the evolution and structure of various genomic elements in *Caenorhabditis* nematodes. Previous comparative genomic studies have investigated multiple *Caenorhabditis*species (Stevens et al. 2019); however, at the time, the genome assemblies for the most polymorphic outcrossing species were fragmented and contained duplications.

We generated a high-quality telomere-to-telomere genome assembly of *C. brenneri*, using both short and long reads and chromosome conformation capture data. We utilized a super-inbred strain, VX0223, which was created by 300 generations of inbreeding. We performed repeat masking and annotated the genome using bulk RNA-seq and full-length transcript sequencing. Then we conducted a comparative analysis of various genetic features, including gene number, intron number and lengths, the number of repetitive elements and repeat-family sizes, as well as the evolution of gene-family sizes and changes in their genomic co-localizations, across *Caenorhabditis* species with varying population sizes, *C. elegans*, *C. remanei*, and *C. brenneri,* using corresponding chromosome-level genome assemblies. Our findings contribute to a better understanding of the complex interplay between population size and the evolution of genomic elements in nematodes and augment the earlier findings.

536V **Natural polymorphisms in the GTPase-activating protein GAP-2 underlie correlated variation in foraging behavior and the dynamic neuron-specific expression of DAF-7** Harksun Lee[1], Sonia Boor[1,2], Zoë Hilbert[2], Josua Meisel[2], Ye Wang[3], Erik Andersen[3], Sylvia Fischer[1], Dennis Kim[11]Pediatrics, Harvard Medical School, [2]Biology, MIT, [3]Northwestern University

Defining polymorphisms in wild populations that influence behavior can provide insight into the molecular changes underlying natural genetic variation in behavior. We have carried out a molecular analysis of natural variation in the neuron-specific expression of *daf-7*, encoding a TGF-beta ligand that controls diverse behaviors of *C. elegans*. We identified a natural polymorphism affecting an alternatively spliced isoform of *gap-2*, encoding a GTPase-activating protein member of the SynGAP family. We determinted that the *gap-2* variants modify signaling from the ADE pair of neurons to activate *daf-7* expression in the ASJ pair of sensory neurons and cause animals to shift from a predominantly exploitation behavior, dwelling, to an exploratory behavioral state, roaming. Our data illustrate how transcriptional state variation in the expression of a single gene in a pair of neurons can be correlated with behavioral state variation in natural populations.

537V **Interaction of genetic variation and diet on stress resistance in *Caenorhabditis tropicalis* isolates** Tzitziki Lemus[1], Leonid Kruglyak[21]University of California Los Angeles, [2]Human Genetics, Howard Hughes Medical Institute

The gut microbiome influences many of its host traits. In humans, disruption of the microbiota balance has been associated with various diseases including obesity, metabolic syndrome, and autoimmune disorders. However, it is challenging to study the mechanisms by which bacteria influence their human hosts due to the complexity of bacterial communities and the genetic diversity of humans. The nematode *Caenorhabditis elegans* has recently been used as a model organism to study the influence of the microbiome and diet on several phenotypes. Studies have shown that the worm microbiome/diet affects important traits such as development, life span, metabolism, and resistance to chemotherapy drugs, but the underlying mechanisms are not well understood.

I recently discovered that resistance to cold stress in a related nematode, *Caenorhabditis tropicalis*, is affected by the worm diet. Interestingly, different *C. tropicalis* isolates are differently affected depending on their diet. Isolate JU1639 was highly susceptible to cold stress when grown on *E. coli* HT115 but survived when grown on OP50, whereas JU1373 worms die regardless of their bacteria diet. Genetic analysis suggests that cold stress resistance is a dominant trait, and initial mapping reveled a potential QTL on chromosome III. Currently, I am working on refining and validating the QTL associated with cold stress resistance, making use of CIMR, and in dissecting the bacterial elements and pathways involved in the cold stress survival difference. The genetic variants uncovered by this study will further our understanding of the mechanisms by which diet and microbiome modulate an organism›s phenotype, and how this modulation depends on the host genetic variation.

538V **Programmed DNA elimination is the ancestral state in *Caenorhabditis*** Lewis Stevens, Manuela Kieninger, Pablo Gonzalez de la Rosa, Mark BlaxterWellcome Sanger Institute

Programmed DNA elimination (PDE) involves the removal of specific DNA sequences in some cells during development, leading to differences between the germline and somatic genomes. Originally discovered in the animal-parasitic ascaridid nematode family, PDE is now known to occur in a diverse array of eukaryotic lineages, including ciliates and vertebrates. Recently, new sequencing technologies have revealed that PDE occurs in many taxa where it has previously been missed, including in the free-living nematode *Oscheius tipulae*, suggesting PDE may be more widespread than previously thought. Here, we unexpectedly discovered PDE in the early diverging species of the genus *Caenorhabditis*. Using PacBio HiFi long-read and Hi-C data, we reconstructed the germline and somatic genomes of *C. monodelphis* and found that this species undergoes PDE during early embryogenesis, leading to the fragmentation of its six germline chromosomes to form 15 somatic chromosomes. Consistent with findings in Ascarididae, we found that the germline-restricted DNA is enriched for satellite repeats and contains genes with roles in germline function, including *puf-8*, a post-transcriptional regulator that has multiple functions in germline development and has homologs in humans and Drosophila. To understand the evolutionary origins of PDE in *Caenorhabditis*, we generated chromosome-level reference genomes for species across the *Caenorhabditis* phylogeny. Despite being absent in the majority of species, we found that PDE is present in multiple early diverging species, strongly suggesting that PDE is the ancestral state in *Caenorhabditis* and has therefore been lost during the evolution of many *Caenorhabditis* species, including *C. elegans*. Once thought to be an obscure and taxonomically-restricted developmental process, our results suggest that PDE has played an important role during the evolution of germline development in *C. elegans* and many other nematode species.

539V **Manipulating sex determination in *Caenorhabditis* tetraploids to evaluate Haldane's rule** Jonathan Harbin, Abdul Abubaker, Ronald EllisRowan 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 crosses between the closely related nematode species *C. nigoni* and *C. briggsae*, since Woodruff et al observed that crosses between *C. nigoni* males and *C. briggsae* hermaphrodites produced no living males, and that the reciprocal crosses between *C. briggsae* males and *C. nigoni* females produced only sterile males. Despite its broad applicability, there is still lively debate about the factors that underlie Haldane's rule.

The *Caenorhabditis* sex determination cascade is initiated in the early embryo by the ratio between the number of *X* chromosomes and sets of autosomes. Disrupting this cascade by altering the sex determination pathway can drive animals to develop as male regardless of their *X:A* ratio. By manipulating sex determination and ploidy within nematodes we can study whether the hybrid heterozygous sex disadvantage is due to chromosome incompatibles (dominance theory) or male specific gene evolution (faster male theory). To that end, we adapted the Schvarzstein method for producing polyploid *Caenorhabditis* strains. Through inactivation of *rec-8* by RNA interference we produced a *C. nigoni* polyploid strain, a *C. briggsae* tetraploid strain already existed. DAPI staining confirmed that the *C. nigoni* female animals are tetraploid, and most likely *XXX; AAAA*, and the males appear to be *XX; AAAA*. Suggesting the *X:A* ratio for determining sex has shifted during *Caenorhabditis* evolution.

Remarkably, using these tetraploid strains, we can now produce fertile interspecies hybrids. These not only include fertile hermaphrodites, but also healthy fertile males. This result strongly supports the model that Haldane's rule in diploid crosses is caused by incompatibility between the genes on the single *X* (which of necessity comes from only a single species), and

interacting products made by the pairs of autosomes from both species. As a result, negative interactions should be minimized in tetraploid males, which have one *X* from each parent species. We are now studying backcrosses of these hybrids to each parent species. Although the success rate for individual crosses is low, the resulting progeny tend to be healthy and vigorous. We are now utilizing *tra-1* mutations that drive animals to develop as males, regardless of their *X:A* ratio, to study if maleness can drive Haldane's rule in male *XX* hybrids.

540V **How *Caenorhabditis elegans* responds to Candida albicans: from parental attraction to progeny rejection.** Romina E D›Almeida[1], Reeta Rao[21]INSIBIO CONICET, [2]Biology and Biotechnology, Worcester Polytechnic Institute

In its natural habitat, when *Caenorhabditis elegans* consumes harmful bacteria, its intestine is colonized, triggering responses that include the activation of the immune system to clear the infection, and the nervous system to escape from the pathogen, to learn to avoid it the future and, to transmit epigenetically the learned information to the next generations as part of the strategies to improve survival of the progeny.

Using the powerful genetics and simple yet evolutionarily conserved systems of *C. elegans*, we aim to describe the neuronal pathways and behavioral responses as well as the mechanism of transgenerational epigenetic inheritance of exposure to fungi, like the human pathogen *Candida albicans*. Presenting a choice of microbial foods to naïve *C. elegans*, we observed a strong preference for *C. albicans* over the non-pathogenic *Escherichia coli* OP50, or even the pathogenic bacteria *Pseudomonas aeruginosa*. Subsequently, within 4-6 h, *C. elegans* escape the *C. albicans* lawn, in correlation with distension of the anterior part of the nematode's intestine. In addition, the population of *C. elegans* in contact with *C. albicans* for more than 4 h learns to avoid this specific yeast in future encounters, and this learned avoidance behavior is transmitted epigenetically to its progeny for one to four generations. Our findings suggest that the responses to *C. albicans* mimics mechanisms involved after a *P. aeruginosa* infection. Future studies will reveal unique pathways to this eukaryotic pathogen.

541A **Modular safe-harbor transgene insertion for targeted single-copy and extrachromosomal array insertion in Caenorhabditis elegans.** sonia el mouridi, Faisal Alkhaldi, Christian Frøkjær-JensenKAUST

The emergence of CRISPR/Cas has revolutionized many aspects of science. In C. elegans, several different strategies were developed to edit the genome and insert transgenes.

In order to increase the frequency of site-specific single-copy transgene and array insertion, we developed a method named modular safe-harbor transgene insertion (MosTI). Our method was designed to be highly modular: MosTI allows easy conversion between selection markers at insertion site and a collection of universal targeting vectors with commonly used promoters and fluorophores.

Our strategy consists uses CRISPR/Cas9 to cut a landing site and insertions are detected by positive selection using a set of split markers (unc-119, hygroR, and gfp). Thus, only animals with chromosomal insertions are rescued, resistant to antibiotics, or fluorescent, respectively. We have generated three permissive safe-harbor intergenic locations (Chr. I, II, and IV) where transgenes are reproducibly expressed in somatic and germ cells.

Single-copy insertion with MosTI is efficient using either constitutive or heat-shock inducible Cas9 expression (25-75% insertion frequency) and inserts can be generated from a multiplexed injection mix. We also show that extrachromosomal arrays can efficiently be integrated (7-44%) at MosTi landing sites or at the endogenous unc-119 locus. We characterized eight array insertion using two different sequencing methods. We determined the plasmid copy number using short-read sequencing and used long-read sequencing to characterize the structure and size. The eight integrated arrays contained 6-37 plasmid copies and one array integrant, characterized in greater detail, was 5.4 Mb.

Using universal targeting vectors, standardized insertion strains, and optimized protocols, it is possible to construct complex transgenic strains which should facilitate the study of increasingly complex biological problems in C. elegans.

542A **PALS-22 modulates RNAi-directed silencing of repetitive DNA and epigenetic inheritance** Chee Kiang Ewe[1], Oded Rechavi[21]Sagol School of Neuroscience, Tel Aviv University, [2]Tel Aviv University

RNA-based mechanisms are amongst the most ancient and conserved genome surveillance systems. By monitoring the expression of tandemly repeated transgenic arrays in *C. elegans*, we sought to investigate the molecular basis of RNA-based defense against repetitive genetic elements. It was previously found that eliminating *pals-22*, a gene encoding for a protein containing ALS2cr12 signature, led to de-silencing of repetitive DNA. Interestingly, it was recently reported that deleting *pals-22* conferred resistance to microsporadia (eukaryotic intracellular parasite) and viral infection. In this study, we show that knocking out *pals-22* activates RNAi-directed silencing by inducing a pathway homologous to the mammalian RIG-I helicase viral response. Additionally, we find

that *pals-22* mutants fail to maintain RNAi inheritance, unlike wildtype animals. Together, these results demonstrate the impact of small RNA on the epigenetic landscape and the maintenance of genomic integrity.

543A **The E isoform of MEC-2 with a long C-terminal provides mechanosensation** Talia Magdolna Keszthelyi[1,2], Regina Legradi[1,3], Dora Palya[1,3], Kalman Tory[1,31]Pediatrics, Semmelweis University, [2]MTA-SE Lendület Nephrogenetic Laboratory, [3]MTA-SE Lendület Nephrogenetic Laboratory, Semmelweis University

Introduction: The podocin encoding *NPHS2* is the most frequently mutated gene in steroid-resistant nephrotic syndrome. We aimed to generate an *in vivo* model to study the interallelic interactions of podocin. The homologue of *NPHS2* in *C. elegans* is *mec-2* encoding several splice isoforms. Among them, MEC-2A has been considered to be the canonical one. The *mec-2* null-mutant worms are touch insensitive. Recently (*Liang et al. Nucleic Acids Res 2022*), two isoforms, MEC-2A and MEC-2E, were described to function in concert and rescue the touch insensitivity only when expressed together. The MEC-2E isoform, also containing a large C-terminal, is significantly longer than the MEC-2A (1239 vs. 481 AA). They also found the *mec-2* mutants to be insensitive to olfactory stimuli. Our aim was to identify the canonical MEC-2 isoform(s) to set the stage for the animal model of podocin interactions.

Methods: Vectors encoding different MEC-2 isoforms under *mec-2* promoter and a selection marker (*cbr-unc*) were generated with NEBuilder DNA assembly kit. To avoid quantitative differences due to extrachromosomal expression, we implemented the MosSCI (Mos1-mediated Single Copy Insertion) technique to achieve chromosomal integration. The *mec-2* nonsense mutant (Tu37) strain was kindly provided by the laboratory of Prof. M. Chalfie. Worms were transformed by microparticle bombardment or microinjection. For gentle touch test eyebrow hair was used in a blinded experiment. Chemotaxis assay was performed in two experimental designs based on the method described previously by *Margie et al. J Vis Exp 2013*. **Results:** Integrant strains were successfully generated expressing MEC-2A or MEC-2E. While no rescue was achieved by MEC-2A expression, the mechanosensation of the strain expressing the MEC-2E isoform was indistinguishable from that of the wt. Coexpression of MEC-2A in the MEC-2E expressing mutants did not further improve the touch sensation. On the other hand a premature stop in the last third of the C-terminal of MEC-2E (c.3076_3077CC>TG, p. P1026*) abolished the touch sensation. We could not detect a significant loss of function in the *mec-2* null-mutants by the chemotaxis assay.

Conclusion: As the MEC-2E isoform was able to rescue the mechanosensation defect on its own, we conclude that the MEC-2E isoform is the canonical transcript. The large C-terminal of MEC-2E is thus crucial for the mechanosensation function. We detected no relevant olfactory function for MEC-2.

544A **New models of transcriptional adaptation in *C. elegans*** Yuntao Charlie Song, Vahan Serobyan, Didier Stainier Max Planck Institute for Heart and Lung Research

Transcriptional adaptation (TA) is a phenomenon whereby a mutation in one gene triggers the transcriptional modulation of other genes, named adapting genes, independent of protein feedback loops. TA has been observed in multiple species, including zebrafish, mice, and *C. elegans*, suggesting a conserved underlying mechanism. However, the current TA models in *C. elegans* have several limitations, such as the lethality of homozygous mutants and multiple isoforms of the mutant gene. Also, the mutant mRNA degradation factors required for TA diverge between these models. Hence, new models are needed to study TA in *C. elegans*. In this project, we explore the information on Wormbase to find candidate mutant genes. Based on desirable criteria to investigate the mechanisms underlying TA, such as existing full locus deletion (FLD) alleles as well as premature termination codon (PTC) alleles with mild phenotypes, we selected 40 genes for further investigation. We then detected mutant mRNA degradation in the PTC alleles of several genes: *amgo-1*, *tbb-1*, and *maco-1*. Currently, we are searching for adapting genes through RNA-seq on these mutants. Overall, the initial goal is to discover new TA models that will allow further studies on the underlying mechanisms.

545A **Distinct roles for SAM synthases in histone methylation** Alexander Munden, Adwait A Godbole, Amy K WalkerProgram in Molecular Medicine, University of Massachusetts Chan Medical School

S-adenosylmethionine (SAM) is the primary methyl donor in most organisms and is synthesized from methionine generated through the one-carbon cycle (1CC). Alterations in the 1CC have a profound impact on lifespan, development, and stress responses. For example, restriction of carbon intermediates in media or knockdown of SAM synthase results in dampened histone methylation. The formation of SAM is catalyzed by SAM synthase enzymes that are essential for most life. Though some organisms have only a single SAM synthase, both humans and *C. elegans* have multiple SAM synthases that display tissue specificity. To what extent different SAM synthases have on all of histone methylation is unknown. In addition, how these synthases may function in different tissues is not clear.

To address these questions, we have embarked on a systematic analysis of the impact of SAM synthase loss in *C. elegans*. By

knocking down the SAM synthase and probing for different histone marks, we will share the differential impact on histone methylation. Recently, our lab has demonstrated that knockdown of *sams-1* and *sams-4* results in distinct, locus specific changes in H3K4me3. To expand on the tissue-specific effects of SAM synthases and to interrogate these changes in a genome-wide fashion, we have adapted the NuTRAP system (Nuclear tagging and Translating Ribosome Affinity Purification) for use in C. *elegans*. This technology uses self-cleaving peptide tags to self-biotinylate a tagged nuclear pore protein and allow for efficient bead-based purification strategies of a specific cell type. With this work, we will further elucidate the complex manner in which SAM synthases regulate the epigenome in a tissue-specific manner.

546A **High-throughput Screening of RNAi Phenotypes in *C. elegans*** Eleanor C Warren, Karen S Sarkisyan, Andre E.X. Brown
London institute of medical sciences

RNAi provides a valuable technique for identifying loss of function phenotypes in *C. elegans,* and although several large-scale RNAi screens have been carried out, most manually assess phenotypes by eye, limiting throughput and sensitivity. We use a high throughput phenotyping platform to allow in depth assessment of genome wide RNAi knockdowns. Using a high-resolution multi-worm tracker, a RNAi library of 6198 *C. elegans* genes with orthologues in humans can be screened in a single day. From the resulting tracking data, features covering morphology, path, posture, and velocity are extracted enabling quantification of phenotypes. Furthermore, by assessing a strain of *C. elegans* with enhanced neuronal RNAi (pan-neuronally expressed SID-1), behavioural and morphological phenotypes arising from neuronal gene knockdown can be identified. This large-scale quantification of RNAi phenotypes will help to increase our understanding of gene function in *C. elegans.*

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548A **Combining behavioral and high-throughput transcriptional measurements in single individuals for uncovering gene-expression dynamics across development** Nabeel S. Ganem, David S. Scher-Arazi, Sharon Inberg, Shay Stern Faculty of Biology, Technion - Israel Institute of Technology

Capturing transcriptional dynamics across and within developmental stages is crucial for understanding the regulation of gene-expression during these long timescales. However, high-throughput approaches for gene expression profiling during development rely on snapshots of synchronized populations at relatively sparse time-points. In order to reveal the fast and continuous structure of gene-expression dynamics across development, we used a multi-camera imaging system to monitor the development time of multiple single *C. elegans* individuals at high spatiotemporal resolution and under tightly controlled environmental conditions. We then utilized this high-throughput imaging system, by performing RNA deep-sequencing analysis in single animals after identifying their exact time of development at seconds time-resolution. Thus, each worm represents a time-sample of the dynamics of gene expression during development. Sampling of ~200 individuals, homogenously distributed during the 10 hours of the fourth larval stage (L4), allowed us to quantify complex and fast changes (minutes resolution) in gene expression across development. Our measurements reveal thousands of genes which are showing multiple temporal trajectories that are variable in structure. Interestingly, the identified smooth trajectories of multiple gene-expression patterns over minutes timescales, suggest that ergodicity within *C. elegans* populations can recapitulate fast dynamical processes of gene-regulation. Furthermore, we utilized temporal signatures of gene-expression to construct a computational model that predicts the developmental age of individuals, without directly measuring their development time. Overall, our results demonstrate the integration of behavioral and gene-expression quantifications in single animals to reconstruct high resolution temporal patterns of gene-regulation across developmental timescales.

549A **Uncovering genomic drivers of ZGA and early embryonic development in *C. elegans* using single-nucleus multiomics** Ser van der Burght[1,2], Francesco Carelli[1,2], Alex Appert[1,2], Yan Dong[1,2], Matthew Hill[1,2], Stathis Megas[1,2,3,4], John Marioni[3,4,5], Julie Ahringer[1,21]The Gurdon Institute, University of Cambridge, [2]Department of Genetics, University of Cambridge, [3]Cancer Research UK Cambridge Institute, University of Cambridge, [4]European Molecular Biology Laboratory, European Bioinformatics Institute, [5]Wellcome Sanger Institute

As an animal develops from a single transcriptionally inactive zygote, its zygotic genome is activated (ZGA) while maternally deposited RNA and proteins are cleared. Following this maternal to embryo transition, the lineages that give rise to different tissues and organs are specified. In *C. elegans*, ZGA starts in the three somatic blastomeres in the four-cell embryo when TFIID-sequestering-proteins OMA-1 and OMA-2 are degraded, allowing transcription to occur (Seydoux & Fire, 1994, Guven-Ozkan *et al.,* 2008). We aim to find genomic drivers of ZGA and lineage specification by following changes in genome accessibility and transcription from mother to daughter cells during these processes.

We have jointly profiled chromatin accessibility and gene expression in nuclei from collections of 1-50 cell embryos. Following clustering of nuclei and cell-type annotation using marker genes, we then assigned cells to the lineage tree. We found that a set of ~250 genes have high expression in somatic nuclei from the 4-cell stage and persist until ~28-cell stage, depending on

the lineage. Among these are *very early transcript* (*vet*) genes of unknown function (Seydoux & Fire, 1994) and genes encoding transcription factors, SCF complex components, RNA-binding zinc finger proteins, and RNA metabolism regulators, which may play a role in the maternal to embryo transition. We identified several highly enriched motifs in their promoters and are currently investigating which TFs might be driving expression. Additionally, we found thousands of sites accessible in early cells, including P-cells, suggesting a genome that is primed for transcription at the start of ZGA.

Through combining joint profiling of genome accessibility and gene expression with the invariant lineage of *C. elegans*, we are investigating the regulatory events that occur between mother and daughter cells. We observe the sequential opening and closing of chromatin across transcription factor cascades and large-scale waves of genome accessibility and gene expression changes associated with developmental progression. We are currently working to determine the gene regulatory networks underlying these changes. Our study identifies the first steps of how the genome is read to control development.

550A **How is *unc-6/Netrin* regulated and what are its primary roles in establishing various neuronal circuits?** Jonathan D Rumley, Oliver Hobert Biological Sciences, Columbia University

The "Netrin system" has well-studied roles in axon pathfinding and less well-studied roles in synapse formation. I am studying the relative contribution of UNC-6/Netrin derived from multiple neuronal and non-neuronal sources by characterizing the expression and phenotypic effects of *cis*-regulatory alleles of *unc-6* in the nematode *C. elegans*. I hypothesize that deleting different sections of the *cis*-regulatory region of *unc-6* will result in alleles that lose expression in subsets of neurons and non-neuronal cells, depending on the locations of transcription factor (TF) binding sites that regulate expression in these cells. This work will enable me to identify circuit organizer TFs and their binding sites that regulate *unc-6* expression in different cell types and neuronal circuits, and may enable me to distinguish between *unc-6*-expressing cells required for axon guidance and synapse formation. To achieve these goals, I will 1) determine the expression of an *unc-6 gfp* reporter allele generated by CRISPR/Cas9-genome engineering and its dependence on TFs that are known to control the identity of *unc-6* expressing neurons, 2) generate *cis*-regulatory alleles of *unc-6* by CRISPR/Cas9 genome engineering and 3) assess the functional consequence of neuron-type specific expression of *unc-6*. To identify the neurons expressing the *unc-6* reporter in WT and mutant worms, I will use the NeuroPAL system, which enables the unambiguous identification of all *C. elegans* neurons based on the combination of a color code produced by the differential expression of three fluorescent reporters and cell position. To assess functional consequences of neuron-type specific expression of *unc-6*, I will use reagents that enable the visualization of axon pathfinding and synapse formation of neurons to assess the circuit-specific effects of *unc-6 cis*-regulatory alleles on axon pathfinding and synapse formation. Through this work, I will elucidate the regulation of *unc-6*, as well as its function in axon guidance and synapse formation in various neuronal circuits.

551A **Sequencing-based mutagenesis screening of *C. elegans* identifies modifiers of *let23* EGFR signaling** Hillel Schwartz, Paul Sternberg Biology and Biological Engineering, California Institute of Technology

Traditional mutagenesis screens in *C. elegans* have relied on the ability to recover a viable strain containing the mutation either directly from the phenotypic animal (nonclonal screens) or from a population of siblings that include phenotypic animals (clonal screens). We have developed a sequencing-based screening method that combines the screening efficiency of a nonclonal screen with the ability of clonal screens to analyze inviable mutants: rather than recover a mutant strain, phenotypic animals are directly sequenced. Mutations identified as candidates to cause the phenotype can then be introduced using CRISPR and tested for their ability to reiterate the mutant phenotype.

We screened for partial suppression of the early, paralyzed lethality caused by loss of the EGF receptor gene *let23*, recovering animals that were still inviable but that showed increased growth and movement and lived for days longer than did unmodified *let23* mutants. Individual animals were processed directly without amplification to generate sequencing libraries. From 84 animals we recovered usable sequence of 68, and in these animals known mutations could be detected roughly half of the time. We identified more than 20,000 exonic mutations, of which a third were coding changes, homozygous, and consistent with changes typically caused by EMS mutagenesis. Mutations were identified as candidates to be causal for the mutant phenotype if representation of the affected gene was enriched, if the mutations were concentrated in a small region within the gene's structure, or by examination of genes already implicated in the relevant biology. Once candidates had been identified, they were reintroduced to the genome using CRISPR (12 mutations), were obtained as identical nucleotide changes from the Million Mutation Project (5 mutations), or in the case of nonsense mutations were tested using available lossoffunction alleles (3 genes).

From this we have identified eight genes, implicated by 27 mutations in 25 different animals, that mutate to delay the lethality caused by loss of *let23*. One of these is the *Ras* homolog *let60*, mutated at a position implicated in human cancer but not previously studied in *C. elegans*. The other seven loci are novel modifiers of *let23* signaling in *C. elegans*; of these seven, six have identifiable human homologs, at least three of which are implicated in epithelial cancer.

552A **UNC-13 microexon alternative-splicing regulators and its functional relevance in *C. elegans*** Bikash Choudhary, Rebekah N Jameson, Adam D NorrisSouthern Methodist University

Alternative splicing (AS) is one of the phenomena to generate transcriptomic and proteomic diversity in higher eukaryotes. Exon sizes ranging from 3-30 nucleotides (nts) are classified as microexons (µexons). µexons are shown to be enriched in the nervous system of higher model organisms and their altered splicing leads to autism-related neurological disorders. Using *C. elegans* as a genetic model, we found a spliceosomal component, *prp-40*, as the master regulator of µexon AS. To find additional regulators,we used an alternatively-spliced 9nts µexon of UNC-13, a pre-synaptic protein, as a µexon model. An UNC-13 µexon based bicolor minigene reporter transgenic animal showed a strictly regulated splicing event in different neuronal subtypes in the nervous system. We also found *exc-7*/*Elav* and *mbl-1*/*MBNL-1* RNA binding proteins (RBPs) differentially regulates inclusion of this UNC-13 µexon. *C. elegans* ventral cord (VC) motor neurons invariably showed µexon-included UNC-13 (UNC-13_included) isoform, whereas a set of olfactory neurons showed µexon-skipped (UNC-13_skipped) isoform. To understand the physiological relevance of the two different isoforms, we generated strains expressing either of the isoforms (UNC-13_skipped and UNC-13_included) using CRISPR-based genome editing. UNC-13_skipped animals showed locomotory deficits, possibly by altering the release of synaptic vesicles (SVs) at synapses. On the contrary, UNC-13_included animals showed olfactory deficits. In conclusion, we found a set of regulatory candidates of µexon-splicing and functional consequences of altered splicing using UNC-13 µexon as an example.

553A **Heterochromatin formation in embryogenesis at single nucleus resolution** Toby Buttress, Julie Ahringer Gurdon Institute, University of Cambridge

Heterochromatin is essential for silencing of repetitive elements and for organization of higher order genome architecture. In animal development, EM dense heterochromatin is initially absent, and so repressive chromatin modifications must be *de novo* re-established to ensure a return to normal genome regulation. In *C. elegans*, H3K9me2 is undetectable in 1-cell embryos whilst H3K9me3 is present at low levels (Mutlu *et al*. 2018 *Sci. Adv.*). Both heterochromatin marks increase through embryogenesis, however a precise description of their deposition is lacking. Furthermore, the mechanism by which heterochromatin re-establishes at specific genomic locations is largely unknown. Here we utilize super resolution Stimulated Emission Depletion (STED) microscopy to examine the repressive modifications H3K9me2 and H3K9me3 through embryo development. STED microscopy reveals heterochromatin formation dynamics at 20 nm resolution, a ten-fold increase in clarity compared to confocal imaging. H3K9me2 forms distinct puncta of ~120nm that gradually increase in number and then become enriched at the nuclear periphery, whereas H3K9me3 forms larger domains. H3K9me2 and H3K9me3 are present in the same nuclear territories but largely do not colocalize. To identify the genomic features first targeted for heterochromatin formation, we are developing a method to simultaneously profile gene expression and histone modifications in single nuclei of early embryos, by co-opting the 10x Genomics multiomics ATAC reaction with CUT&Tag (Cleavage Under Target and Tagmentation). Collectively, our approaches reveal heterochromatin formation in embryogenesis at unprecedented resolution and will reveal insights into the mechanism by which repressive chromatin marks are targeted for deposition.

554A **SET-24 mediates epigenetic silencing maintenance in the *C. elegans* germline** Chenming Z Zeng[1], Giulia Furlan[2], Jon Price[2], Miguel Almeida[2], Juan Carlos Rueda Silva[2], Meng Huang[3], Shouhong Guang[3], Eric Miska[21]Department of Biochemistry, University of Cambridge, [2]University of Cambridge, [3]University of Science and Technology of China

Non-DNA sequence-based epigenetic information can be inherited over generations, in a process termed as transgenerational epigenetic inheritance (TEI). The nematode *C. elegans* is an ideal model to investigate the molecular mechanisms of TEI, because small RNAs can robustly initiate epigenetic silencing that can be inherited for many generations. Small RNA-driven epigenetic silencing is associated with repressive histone methylation marks, such as H3K9me3 and H3K27me3. SET-24 is a protein containing a conserved SET domain, which exists in several histone methyltransferases, and two SPK domains. SET-24 is expressed in the germline and localizes to nuclei. *set-24* mutants have a mortal germline (Mrt) phenotype, reaching sterility after 5-7 generations, suggesting a role for SET-24 in germline integrity. Interestingly, some *set-24* lines escape sterility, indicative of a role in balancing epigenetic signals. In addition, the inheritance of small RNA-driven epigenetic silencing is compromised in *set-24* mutants. Using quantitative proteomics and Yeast 2-hybrid, we found SET-24 interacts with HCF-1, which is involved in transcriptional regulation and associates with known chromatin remodelling complexes. We conclude that SET-24 alters the status of histone modifications and promotes TEI.

555A **Dissecting the molecular mechanisms of miRNA-binding Argonautes in spermatogenesis: shedding light on unexplored regulators of paternal fertility** Volker Nitschko, Uri Seroussi, Mathias Renaud, Julie M. Claycomb Molecular Genetics, University of Toronto

RNA interference (RNAi) pathways have emerged as conserved and essential regulators for processes like development, fertility,

and epigenetic inheritance. Misregulation has been shown to cause developmental defects and diseases such as cancer. At the core of RNAi are the conserved Argonaute (AGO) proteins acting as the catalytic engines. They are guided to their targets by small RNAs (sRNAs) such as miRNAs.

Most studies have investigated the regulatory role of RNAi/miRNAs during oogenesis and embryogenesis. Given the importance in these processes it is highly likely that miRNA pathways are also crucial regulators of spermatogenesis, for which we so far lack an understanding. Furthermore, there has been a continuous decline in sperm quality in Western men for which we do not understand the causes.

A recent systematic study in our lab has characterized all 19 functional AGOs in *C. elegans*. This study showed RDE-1 and ALG-5 are the only miRNA-binding AGOs expressed during spermatogenesis. Mutations in *rde-1* and *alg-5* lead to stress-induced fertility defects and generally lower brood sizes. This indicates that these AGOs and the associated miRNAs play a crucial role in fertility, including in the regulation of sperm development/differentiation. To test this, we are investigating at which stage(s) of spermatogenesis and sperm development miRNA-AGOs may function by examining mutant germlines for developmental defects or delays. In addition, we are identifying which miRNAs are bound to ALG-5 and RDE-1 in males, L4 hermaphrodites, and adult hermaphrodites to establish sets of gonad specific AGO-bound miRNAs. We expect reduced levels of these miRNAs when sequencing total sRNAs from mutants of the same age/sex. We are using RIP and eCLIP-seq to identify AGO mRNA targets and will cross-reference these data with small RNA seq to identify which miRNA regulate each target.

These studies will help to overcome the knowledge gap about the molecular mechanisms of the miRNA-binding AGOs in spermatogenesis. Many important human miRNAs, spermatogenesis genes, and over 50 % of the sperm proteome are conserved in *C. elegans*. This makes it an ideal model and will allow for our findings to inform future foundational research into spermatogenesis and miRNA pathways in more complex organisms like humans. This work harbors the potential to uncover key pathways and genes that could be exploited for human therapeutics such as male fertility treatments or non-hormonal contraception.

556A **A comparative single-cell neuronal transcriptome for both sexes of *C. elegans*** Rizwanul Haque[1], Ramiro Lorenzo[2], Hagar Setty[1], Yehuda Salzberg[1], Gil Stelzer[3], Patrick Laurent[2], Meital Oren-Suissa[11]Brain Sciences, Weizmann Institute of Science, [2]ULB Neuroscience Institute, Université Libre de Bruxelles, [3]Bioinformatics Unit- Life Science Core Facilities, Weizmann Institute of Science

The two sexes of a given species often exhibit distinct behaviors due to differences in neuronal wiring and molecular signatures. Although sexual dimorphism in the nervous system has been extensively studied, the precise molecular mechanisms driving the development of dimorphic neuronal circuits remain poorly understood. To address this gap in knowledge, we utilized single-cell RNA-sequencing to conduct a comparative molecular analysis of the entire adult nervous system of *Caenorhabditis elegans* for both males and hermaphrodites. Our work offers a comprehensive resource, offering invaluable insights into the varied sex-specific expression patterns of over 10,555 genes spanning 112 distinctly identifiable neuronal cell types in the adult nervous system. Our analysis revealed distinct dimorphic gene expression patterns for multiple gene classes, including transcriptional regulators, neuropeptide signaling genes, G protein-coupled receptors, and other gene families. We ranked the neurons based on their degree of dimorphism and identified many neurons which have never been shown to be sexually dimorphic previously. We validated our results by comparing endogenous expression levels of numerous neuropeptides genes in both sexes by using CRISPR transcriptional GFP knock-in strains. Our comprehensive differential gene expression catalog provides a starting point for further research into the regulatory mechanisms governing dimorphic connections and the cellular networks connecting neural and sexual identities. Notably, to our knowledge, this study offers the first thorough sex-specific neuronal gene expression atlas for any organism. Finally, this atlas may offer new candidates for the molecular underpinnings of the sex bias commonly observed in many neurological conditions, including Alzheimer›s and Schizophrenia, as well as new avenues for the development of sex-specific treatments.

557A **Homeodomain-interacting protein kinase (HPK-1) maintains neuronal homeostasis during normal aging and systemically regulates longevity from serotonergic and GABAergic neurons** Maria I Lazaro-Pena[1], Adam B Cornwell[1], Carlos A Diaz-Balzac[2], Ritika Das[3], Nicholas Macoretta[3], Juilee Thakar[1], Andrew V Samuelson[11]Department of Biomedical genetics, University of Rochester Medical Center, [2]Department of Medicine, University of Rochester Medical Center, [3]Department of Biology, University of Rochester Medical Center

Aging and the age-associated decline of the proteome is determined in part through neuronal control of evolutionarily conserved transcriptional effectors, which safeguard homeostasis under fluctuating metabolic and stress conditions by regulating an expansive proteostatic network. We have discovered the Caenorhabditis elegans homeodomain interacting protein kinase (HPK-1) acts as a key transcriptional effector to preserve neuronal integrity, function, and proteostasis during aging. Loss of *hpk-1* results in drastic dysregulation in expression of neuronal genes, including genes associated with neuronal aging. During normal

aging *hpk-1* expression increases throughout the nervous system more broadly than any other kinase. Within the aging nervous system, hpk-1 is co-expressed with key longevity transcription factors, including *daf-16* (FOXO), *hlh-30* (TFEB), *skn-1* (Nrf2), and *hif-1*, which suggests *hpk-1* expression mitigates natural age associated physiological decline. Consistently, pan-neuronal overexpression of *hpk-1* extends longevity, preserves proteostasis both within and outside of the nervous system, and improves stress resistance. Neuronal HPK-1 improves proteostasis through kinase activity. HPK-1 functions cell non-autonomously within serotonergic and GABAergic neurons to improve proteostasis in distal tissues by specifically regulating distinct components of the proteostatic network. Increased serotonergic HPK-1 enhances the heat shock response and survival to acute stress. In contrast, GABAergic HPK-1 induces basal autophagy and extends longevity. Our work establishes *hpk-1* as a key neuronal transcriptional regulator critical for preservation of neuronal function during aging. We are currently looking at the gene expression changes caused by HPK-1 overexpression in serotonergic and/or GABAergic neurons, and looking at the transcription factors responsible for these changes. This will provide some insight into how this kinase and transcriptional cofactor preserves the neuronal proteome during aging and in response to stress.

558A **Altering sequence features of miRNA duplexes influences strand selection and reveals potential imbalances in the relative stabilities of each miRNA strand** Jeffrey C Medley[1], Ganesh Panzade[2], Joel Sydzyik[1], Sarah Coffey[1], Mira Bhandari[3], Anna Zinovyeva[41]Division of Biology, Kansas State University, [2]Department of Health Management and Informatics, University of Missouri, [3]University of Michigan, [4]Kansas State University

Gene expression must be tightly regulated to maintain cellular homeostasis and ensure proper animal development. microRNAs (miRNAs) play a central role as regulators of gene expression by repressing their target genes. A critical step in miRNA-mediated gene regulation is the processing of miRNA precursors into duplexes that comprise two strands. One dominant (guide) strand is loaded into an Argonaute protein to form the miRNA-induced silencing complex (miRISC), while the other (passenger) strand is degraded. However, either strand can be functional if loaded into Argonaute. As each miRNA strand would have different molecular targets, the decision of which strand is loaded into Argonaute effectively determines the target repertoire of miRISC. Previous studies have suggested that 5' nucleotide identity and thermodynamic asymmetry of miRNA duplexes is sufficient to explain strand selection *in vitro.* However, it remains unclear whether those sequence cues play a role in determining strand selection *in vivo*, as many miRNAs appear to have unfavorable sequence features. Here, we used genome editing to alter the sequences of miRNA duplexes to examine the mechanisms of strand selection *in vivo*. We found that mutating 5' nucleotide or thermodynamic asymmetry resulted in distinct effects on strand selection for different miRNAs. For example, mutating the 5' nucleotide of the guide strand to an unfavorable nucleotide was sufficient to reverse strand selection of miR-1 and miR-2 but not miR-58. Our results suggest that a complex interplay of duplex features, which is dependent on the specific sequence of individual miRNAs, may govern strand selection. Further, our analysis revealed unexpected effects on the potential relative stabilities of each miRNA strand. When strand selection of miR-2 was reversed, the levels of the passenger strand were substantially higher than what was observed for the canonical guide strand. Conversely, when miR-1 strand selection was reversed, the levels of the passenger strand remained extremely low. This might indicate differences in miRNA processing or the relative stability of each strand originating from the same duplex. Interestingly, we observed embryonic lethality in miR-58 strand switching mutants, possibly due to aberrant passenger strand accumulation. Collectively these findings provide important insights into how miRNA strand selection is specified *in vivo*.

559A **Noncanonical germline RNA expression from multi-copy transgenes** Maya Spichal, Craig Cameron MelloRTI, University of Massachusetts Chan Medical School

In several organisms, including *C. elegans,* genes with high copy numbers are silenced especially in the germline, presumably reflecting a mechanism that detects and silences self-replicating DNA elements. However, gene copy-number silencing does not occur through the canonical dsRNA, RNAi mechanism, and does not depend on piRNAs, so whether or how small RNAs direct the silencing of high copy number DNA is not known. While using RNA FISH to study events that occur during the silencing of neuronal GFP expressed from different high copy number integrated transgenes, we were surprised to find abundant RNA FISH signals in the late pachytene germline, where the corresponding endogenous gene is not expressed. Further analysis revealed that these RNA signals localize in mutator foci. Small RNA sequencing revealed an abundance of small RNAs covering both the coding and the non-coding regions of the transgene DNA, including the entire plasmid backbone. The small RNAs were predominantly 22Gs and could be found in both the sense and anti-sense direction.

Introduction of a *nrde-2* mutation caused a marked increase in nuage-localized RNA FISH signals but GFP expression was not detected in the germline, suggesting that the increased RNA FISH signals in nuage represent templates for small RNA amplification. Consistent with this idea, mutation of *rde-3,* which is required for template production and small RNA amplification caused release of RNA from nuage into the cytoplasm, however no GFP expression was detected in germ cells.

We also performed RNA FISH to detect anti-sense GFP transcripts. Interestingly, anti-sense transcripts co-localized with nuclear

transcription sites but were not detected in mutator foci. Injection of a promoterless GFP PCR product containing introns but lacking both a translation start and stop codon resulted in high copy transgenes that produced and exported GFP RNA out of the nucleus.

Our results indicate that repeat sequences are transcribed in both strands and that the presence of introns within the resulting non-canonical transcripts promotes export to nuage. We wonder whether perhaps all DNA is transcribed at low levels during pachytene and the presence of high copy numbers makes these otherwise rare RNA signals detectable. We are attempting to address these and other possibilities that could explain how gene copy number is sensed, and we are wondering if there are other potential roles for this mode of transcription in genome surveillance.

560A **Characterization of *Caenorhabditis elegans* F07A5.4, an ortholog of human Olfactomedin 1** Karunambigai S Kalichamy, Martin L Hudson Molecular and cellular biology, Kennesaw State University

Accurate control of nervous system development is critical for normal brain patterning, and defects in this process can lead to neurological disorders such as schizophrenia and Autism Spectrum Disorder (ASD). The transcription factor neurogenin is necessary for the development of neural subtypes and is deeply conserved across species. Despite its importance in neural development, the transcriptional targets of neurogenin are poorly understood, creating an imperative for further study. Previous work from our lab revealed that *C. elegans* neurogenin *ngn-1* plays a role in nerve ring architecture, and neural cell fate specification. In addition, *ngn-1(ok2200)* mutants have an array of neuromuscular defects such as sluggish, uncoordinated movement and precocious egg laying. To help identify downstream targets of *ngn-1*, we performed a comparative transcriptome on messenger RNA isolated from wild type and *ngn-1* mutants. We discovered that F07A5.4 transcript levels are significantly lower in *ngn-1* mutants. F07A5.4 is an ortholog of Human olfactomedin (Olfm1), which is a secreted glycoprotein in the conserved olfactomedin family (OLF). In rodents, OLF domain proteins play important roles in neurogenesis, neural crest formation, and cell adhesion. We hypothesize that F07A5.4 is required for establishing normal nervous system architecture in the worm. To investigate this, we have identified alleles from the Million Mutation Project that mutate conserved amino acids in the F07A5.4 protein sequence. This project aims to characterize the phenotype of those alleles and to establish the normal function of F07A5.4 in *C. elegans* nervous system development.

561A **Understanding the chemical kinetics of auxin-inducible degradation (AID) across TIR1 and degron tag variants** Jeremy Vicencio[1], Daisuke Chihara[1], Nicholas Stroustrup[1,21]Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), [2]Universitat Pompeu Fabra (UPF)

The auxin inducible degron (AID) allows the quantitative spatio-temporal control of protein degredation in *Caenorhabditis elegans*. Our study aims to better understand the kinetics of AID-mediated protein degradation by independently modulating its individual components. By varying the expression levels of TIR1 and the structure of the degron tag, we find that the efficiency of protein depletion is directly related to the strength of the promoter and the number of degron epitopes. Our findings can then be used to achieve the desired level of penetrance of the phenotype of interest.

The AID system has been used to limit protein degredation to specific tissues by expressing TIR1 under tissue-specific promoters. We expand this approach by developing a “dual-channel” AID system that permits the independent control of protein degradation simultaneously in two different tissues. We hypothesize that independently modulating the levels of the same protein in two different tissues can result in a continuum of changes both at the population (lifespan) and molecular (gene expression) levels. In summary, we establish that the AID system can be used not only as an on/off switch for protein degradation, but also, as a finely tunable tool that facilitates varying degrees of protein depletion in different tissues.

562A **Misregulation of mitochondrial 6mA promotes the propagation of mutant mtDNA and causes aging in *C. elegans*** Anne Hahn, Grace Ching Ching Hung, Daniel Campbell, Arnaud Ahier, Rachel Lee, Chuan-Yang Dai, Ina Kirmes, Steven Zuryn Queensland Brain Institute - University of Queensland

In virtually all eukaryotes, the mitochondrial genome (mitochondrial DNA, mtDNA) encodes proteins necessary for oxidative phosphorylation (OXPHOS) and the RNA machinery required for their synthesis inside the mitochondria. Appropriate regulation of mtDNA copy number and expression is essential for ensuring the correct stoichiometric formation of OXPHOS complexes assembled from both nuclear- and mtDNA-encoded subunits. Mechanisms of mtDNA regulation are not completely understood but are essential to organismal viability and lifespan. Here, using multiple approaches, we demonstrate the presence of N6-methylation (6mA) on the mtDNA of diverse animal and plant species. Importantly, we further demonstrate that this modification is regulated in *C. elegans* by the DNA methyltransferase DAMT-1, and DNA demethylase ALKB-1, which localise to mitochondria. Mis-regulation of mtDNA 6mA through targeted overexpression of these enzymatic activities inappropriately alters mtDNA copy number and expression, impairing OXPHOS function and resulting in increased oxidative stress as well as shortened lifespans. Compounding defects in mtDNA regulation, reductions in mtDNA 6mA methylation promotes the propagation of a deleterious

mitochondrial genome within and across generations. Together, these results reveal that mtDNA 6mA is highly conserved among eukaryotes and regulates lifespan by influencing mtDNA copy number, expression, and heritable mutation levels *in vivo*.

563A **CSR-1 RNA interference pathway restricts holocentromere protein CENP-A/HCP-3 localization in Caenorhabditis elegans** Charmaine Yan Yu Wong, Karen Wing Yee YuenSchool of Biological Sciences, The University of Hong Kong

CSR-1, an argonaute of a worm-specific RNA interference pathway, is important for chromosome segregation. Depleting CSR-1 in *C. elegans* slows down mitotic spindle pole separation in a kinetochore-dependent manner, which hints that kinetochores are misattached to the microtubules. HCP-3, the centromeric histone variant, is the homolog of CENP-A in *C. elegans.* Absent of CSR-1 promotes chromatin level of HCP-3 without significantly changing its RNA and protein level in the embryos. When stretching chromatin into long fibers, brighter HCP-3 signal resolves into denser HCP-3 foci in CSR-1-depleted embryo compared to the untreated control. The increase in HCP-3 chromatin deposition on chromatin after CSR-1 depletion is partially independent of the known HCP-3 loading factors, KNL-2 and LIN-53, suggesting a non-classical, improper HCP-3 loading pathway. Negative regulation of HCP-3 holocentromere loading by CSR-1 required its slicer activity and the b isoform. CSR-1 may acts as an HCP-3 deposition repressor, shedding light on the role of RNAi pathways in specifying the localization of centromere proteins on holocentromeres.

564A **The clock gene homolog *Ror/nhr-23* generates both 8-hour molting rhythm and 24-hour circadian rhythm.** Shingo Hiroki[1], Yuichi Iino[2], Hikari Yoshitane[11]Tokyo Metropolitan Instituite of Medical Sciences, [2]University of Tokyo

Animals have internal clocks that generate biological rhythms. In mammals, clock genes such as *Period* constitute the circadian clock with approximately 24-hour period. In C. elegans, the clock genes constitute “the molting clock”, which oscillates with approximately 8 h period during larval development to determine the timing of molting. This implies that the ancestral circadian clock system has evolved into the ‘developmental clock’ in C. elegans. However, the existence of circadian rhythms has also been reported in adult C. elegans, albeit relatively weak. If the conserved clock genes are used to time the molting rhythm with the shorter period, how is the rhythm with the longer period generated?

Here, we reanalyzed circadian transcriptome datasets to answer this question:

1. adult circadian transcriptome (Linden et al., 2010), and 2. L3-L4 developmental transcriptome (Hendricks et al., 2014).

We found that the genes transcribed by NHR-23, a C. elegans homolog of the mammalian clock gene ROR, oscillate with approximately 24-hour periods in adulthood, whereas they oscillate with approximately 8-hour periods during postembryonic development. Furthermore, while previous studies have demonstrated that nhr-23 is necessary for molting during postembryonic developments, we revealed that adult-specific nhr-23 knockdown depletes the circadian transcriptional rhythm.

These results indicate that nhr-23 can generate biological rhythms with different periods, suggesting that the ancestral circadian clock system may have evolved into the multifunctional clock system, rather than a mere clock with a shorter period.

565A **How does RNA polymerase III promote longevity?** Yasir Malik[1], Rene Rivera[1], Yavuz Kulaberoglu[2], Gillian Borland[3], Colin Selman[3], Nazif Alic[2], Jennifer Tullet[11]School of Biosciences, University of Kent, [2]Institute of Healthy Ageing, UCL Department of Genetics, Evolution & Environment, [3]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow

Transcription in eukaryotic cells is carried out by three RNA polymerases. RNA polymerase I synthesises most ribosomal RNAs while RNA polymerase II transcribes mRNAs and miRNAs. The largest and most complex of the three RNA polymerases is RNA polymerase III (Pol III) - specialised to synthesise a variety of non-coding RNAs including the highly transcribed tRNA and 5S rRNA. Recently, Pol III inhibition has been shown to extend lifespan in yeast, worms and flies (Filer *et al* ., Nature 2017).

Here we show that the long life induced by Pol III knockdown leads to dampened protein synthesis and increased tolerance to proteostatic stresses. We find that in both *C. elegans* and *D. melanogaster*, Pol III downregulation leads to transcriptional reprogramming by altering the transcriptional profile of multiple small RNAs, predominantly tRNAs.

Importantly, Pol III inhibition in early adulthood leads to an upregulation in the Unfolded Protein Response (monitored by HSP-4::GFP levels) in Endoplasmic Reticulum (UPR-ER). The UPR-ER is an evolutionarily conserved adaptive regulatory pathway that alleviates protein-folding defects in the endoplasmic reticulum. We present work linking dysregulation of small RNAs, to protein translation and proteostasis with lifespan and stress resistance.

566A **Genetic Interactions Between the Gut Microbiota and *C. elegans* Intestinal Cells** Jessica Hill, Andrew Moore BMB, Colorado State University

The human gastrointestinal tract is home to trillions of microorganisms that dynamically comprise our gut microbiome. Alteration to our gut microbial community leads to disease. At the forefront of host-microbe interactions are intestinal cells, which help establish and maintain the beneficial symbiotic relationships we develop with our gut microbiota. *However, how intestinal cells differentiate between symbionts and non-symbionts is unknow. Furthermore, which intestinal cell responses promote symbiont selection within the gut remains unclear.* We leverage the tractability and simplicity of *Caenorhabditis elegans* to address these questions using a genetics approach. Here, we generated intestine specific transcriptome profiles in response to *E. coli* (OP50), *Pseudomonas aeruginosa* (PA14), and the CeMbio community. To make this intestine specific dataset, we developed a hand dissection method allowing us to isolate the intestines of worms for downstream sequencing assays (i.e., RNA-seq, 16S rRNA gene sequencing). To afford our investigation single cell resolution, we are also working towards generating an intestinal cell specific dataset for the above ecological contexts. We hypothesize that intestinal cells display unique gene expression patterns due to their spatial arrangement and sub-functionalization, allowing for distinct interactions with gut bacteria. These specific interactions are likely required for symbiont recognition and selection. Though we are waiting for the sequencing data to come back, we expect to observe region specific specialization in intestine programs like metabolism and immune function. When completed, these datasets will form a transcriptional atlas of the *C. elegans* intestine. We also expect that these spatial sub-functions within the intestine will govern bacterial colonization. Specifically, we hypothesize that bacterial colonization patterns are regulated by distinct intestinal cell responses. To address this, we transformed fluorescent reporters into OP50, PA14, and individual CeMbio community members. We are in the process of establishing baseline colonization patterns and characteristics for these bacterial groups. From data collected thus far, we have started evaluating the spatial frequency of bacterial colonization throughout the intestine. Overall, our investigations will clarify how intestinal cells interact with and select for gut bacteria, providing insight into microbial community assembly within the gut.

567A **Polymorphic short tandem repeats and their impacts on gene expression variation in *Caenorhabditis elegans*** Gaotian ZHANG[1,2], Ye Wang[1], Erik Andersen[11]Northwestern University, [2]Institut de Biologie de l'École Normale Supérieure (IBENS)

Short tandem repeats (STRs) represent an important class of genetic variation that can contribute to phenotypic differences. However, the diversity of STRs and their impacts on phenotypic variation are still not well understood in many organisms. We recently characterized the distribution of 31,991 STRs with motif lengths of 1-6 bp in the reference genome of *Caenorhabditis elegans*. Of these reference STRs, 27,636 varied in length and/or composition across 540 wild strains from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR). Compared to the reference, polymorphic STRs showed more contraction than expansion. We found that STRs with different motif lengths were enriched in different genomic features, among which coding regions showed the lowest STR diversity and had higher levels of constraint. STR diversity also showed similar genetic divergence and selection signatures among wild strains as in previous studies using single-nucleotide variants (SNVs).

Leveraging the recently generated expression data among wild *C. elegans* strains, we conducted a genome-wide analysis of how STRs affected gene expression variation. We identified thousands of expression STRs (eSTRs) showing regulatory effects and demonstrated that they explained missing heritability beyond SNV-based expression quantitative trait loci. We illustrated specific regulatory mechanisms such as how eSTRs affected splicing sites and alternative splicing efficiency. We also showed that differential expression of antioxidant genes and oxidative stresses might affect STR mutations systematically using both wild strains and mutation accumulation lines. Overall, our results delineate the first large-scale characterization of STR variation in wild *C. elegans* strains and highlight the interplay between STRs and gene expression variation.

568A **Natural genetic variation in multigenerational non-genetic phenomena in *C. elegans*** Marie Saglio[1], Lise Frézal[2], Luke Noble[1], Gaotian Zhang[1], Mohammed Al Johani[3], Christian Frøkjær-Jensen[3], Marie-Anne Félix[11]Institut de Biologie de l'ENS (IBENS), [2]Institut Pasteur, [3]King Abdullah University of Science and Technology

The main mode of biological heredity is based on the DNA sequence. However, recent molecular work in various organisms including *C. elegans* has shed light on other, generally less stable, modes of inheritance. Here we test whether and how such non-genetic inheritance systems are modulated by natural genetic variation, using two multigenerational assays: 1) the mortal germline (Mrt) phenotype and 2) memory of RNA interference.

The Mrt phenotype is a progressive onset of sterility over generations. In the N2 background, mutations in loci affecting the inheritance of small RNAs and histone modifications display a temperature-sensitive (ts) Mrt phenotype. Some *C. elegans* wild isolates surprisingly also display a strong ts Mrt phenotype. Using a cross between two isolates, we previously identified a causal polymorphism in *set-24*, which turned out to be a rare allele (Frézal et al. 2018). To identify more common polymorphisms, we performed a genome-wide association test with 115 isolates, using the number of generations to sterility at 25°C as a quantitative trait. We detected a significant association on chromosome III, which we confirmed using introgression lines from the Mrt isolate JU775 in non-Mrt genetic backgrounds. Further recombinant lines narrowed down the interval to 4.66-6.49 Mb.

Our results show that a seemingly deleterious genotype is maintained at intermediate frequency in the species. This surprising fact may be explained by an environmental rescue. Indeed, naturally associated bacteria and microsporidia suppressed the Mrt phenotype of wild isolates - as well as that of *nrde-2*, *set-2* and *set-24* mutants. Importantly, we revealed a positive, condition-dependent effect of gut-infecting microsporidia on germline maintenance.

In addition to the Mrt phenotype, we directly assayed small RNA inheritance. We independently introduced two germline-expressed GFP transgenes in a set of wild isolates and assayed the memory of a GFP RNAi trigger. While some isolates consistently showed a long memory over generations in the absence of the RNAi trigger (e.g. EG4725), others displayed an intermediate memory (N2), a very short memory (JU775), or no memory (JU1171).

Overall, we show that multigenerational memory is modulated by natural genetic variation in *C. elegans*. The duration of multigenerational memory may thus evolve under natural selection.

569A **Epigenetic inheritance of longevity and diminished telomeric foci in progeny of *pot-1* mutants** Shawn Ahmed, Benjamin McCarthy, Evan Lister-Shimauchi Genetics, UNC Chapel Hill

The Protection Of Telomeres 1 (POT1) protein interacts with single stranded DNA at chromosome termini. *C. elegans* POT1 homologs POT-1 and POT-2 form abundant nuclear foci at telomeres in adult germ cells. However, POT-1 and POT-2 foci vanish from telomeres in 1 cell embryos and gradually accumulate during wild type embryonic development (1). *pot-1* mutants completely lack POT-1 and POT-2 at telomeres in both germ cells and embryos. Gametes of *pot-1* mutants create F1 and F2 embryos that also lack POT-1 and POT-2 in germ cells and embryos, even though the F1 and F2 embryos possess wild type POT-1. The absence of telomeric POT-1 and POT-2 foci in cross progeny of *pot-1* mutants represents the first example of multigenerational epigenetic inheritance in the field of telomere biology, where a phenotype is inherited for more than one generation in the absence of the initiating trigger.

Traits that are epigenetically transmitted for multiple generations are hypothesized to improve fitness of future generations. Consistently, we found that *pot-1* mutants and their F1 cross progeny that lack POT-1 and POT-2 at telomeres display moderately extended lifespan. The transcription factor DVE-1 physically interacts with *C. elegans* telomere binding proteins (2) and promotes several *C. elegans* longevity pathways (3,4). We found that DVE-1 colocalizes with telomeric POT-1 foci in early embryos and in nuclei of some adult somatic cells. To test the role of DVE-1 in *pot-1* mutant longevity, we generated a putative null *dve-1* mutation which resulted in L1 arrest and severe defects in intestinal development. This precludes us from using a null *dve-1* mutation to determine epistasis with respect to the *pot-1* longevity phenotype. Our long-term goal is to understand how epigenetic inheritance of low levels of single-stranded telomere binding proteins at telomeres promotes longevity. This may be important because human telomere length can be epigenetically modulated by psychosocial stress, although the mechanism by which this occurs remains unclear.

1) Lister-Shimauchi et al., *Communications Biology* 2021.

2) Dietz et al., *Nature Communications* 2021.

3) Tian et al., *Cell* 2016.

4) Lan et al., *Cell Reports* 2019.

570A **A circadian-like gene network regulates heterochronic miRNA transcription in *C. elegans*** Brian Kinney[1], Shubham Sahu[2], Natalia Stec[1], Kelly Hills-Muckey[1], Dexter Adams[1], Jing Wang[1], Matt Jaremko[1], Leemor Joshua-Tor[1], Wolfgang Keil[2], Christopher Hammell[11]Cold Spring Harbor Laboratory, [2]Curie Institute

Developmental robustness relies on precise control of the timing and order of cellular events. In *C. elegans*, the invariant sequence of post-embryonic cell fate specification is controlled by oscillatory patterns of heterochronic microRNA transcription that are phase-locked with the larval molting cycle. How these transcriptional patterns are generated and how microRNA dosage is controlled is unknown. Here we show that transcriptional pulses of the *lin-4* heterochronic microRNA are produced by two nuclear hormone receptors, NHR-85 and NHR-23, whose mammalian orthologs, Rev-Erb and ROR, function in the circadian clock. While Rev-Erb and ROR play antagonistic roles in regulating once-daily transcription, we find that NHR-85 and NHR-23 bind cooperatively as heterodimers to *lin-4* regulatory elements to induce a single brief pulse of expression during each larval stage. We demonstrate that the timing and duration of *lin-4* transcriptional pulses are programmed by the phased overlap of NHR-85 and NHR-23 protein expression and that these regulatory interactions are post-transcriptionally controlled by LIN-42, the circadian Period ortholog in *C. elegan*s. These findings suggest that an evolutionary rewiring of the circadian clock machinery is co-opted in nematodes to generate periodic transcriptional patterns that define cell fate progression.

571A **The *Caenorhabditis* Natural Diversity Resource (CaeNDR): A powerful platform for comparative genetics and genomics across the three selfing species** Erik AndersenMolecular Biosciences, Northwestern University

Most *Caenorhabditis elegans* research focuses on the laboratory-adapted strain, N2, limiting the applicability of discoveries to just one strain in the species. We require deeper investigations of traits across the species to understand evolutionary processes and broad conservation to other species. Over the last twenty years, studies of *C. elegans* natural diversity have identified more than 40 different molecular mechanisms underlying differences in this species. The *C. elegans* Diversity Resource (CeNDR) helps to facilitate these studies by providing cryopreserved wild strains, whole-genome sequence and variant data, and a variety of tools to aid quantitative and population genetics. In an effort to create an unparalleled platform for comparative studies across the three selfing *Caenorhabditis* species (*C. briggsae*, *C. elegans*, and *C. tropicalis*), CeNDR evolved into the *Caenorhabditis* Natural Diversity Resource (CaeNDR). First, we amassed, organized, and cryopreserved all of the known natural strains for each of the three species (1,681 *C. briggsae*, 1,385 *C. elegans*, and 681 *C. tropicalis* strains). Then, we created new reference genomes and gene models for *C. briggsae* and *C. tropicalis* to enable comparative genomics and genome resequencing. Then, we collected whole-genome sequence data, called variants, and classified the predicted effects of these variants. Last, we ported tools from *C. elegans* to the other two species. This newly created *Caenorhabditis* Natural Diversity Resource (CaeNDR) allows investigators to investigate natural differences in quantitative traits and discover how molecular mechanisms have evolved across all three species.

572A **Worm Cool Kit: *C. elegans*-specific online tools for single-guide CRISPR planning and gene conservation evaluations** Anat Nitzan[1], Sapir Shemesh[2], Ronen Zaidel-Bar[31]Tel Aviv University, [2]Human Genetics, Tel Aviv University, [3]Cell and Developmental Biology, Tel Aviv University

The number of genetic variations associated with human diseases is increasing rapidly. However, most of these polymorphisms are purely correlative and do not contribute to our understanding of disease etiology or expedite treatment. To facilitate the use of *C. elegans* as a model for human genetic disease we launched the Worm Cool Kit website: http://www.wormcoolkit.com. This website offers four tools: **Orthologs finder** - finds *C. elegans* orthologs to the human genes of interest (and vice versa) by extracting candidates from the OrthoList2 database and then carrying several filtration steps, aimed to increase the likelihood that candidates are true orthologs. **The amino acid conservation tool** - checks if a specific amino acid in a human gene is conserved in the *C. elegans* ortholog. If so, the corresponding site in the amino acid sequence of the worm protein will be indicated. **Automated CRISPR planner for point mutations** – once the user types in the gene and the amino acid to be mutated, this tool provides multiple RNA guides to choose from and, according to the chosen guide, a repair template that contains point mutations to alter the amino acid codon, silent mutations to prevent recutting by cas9 and silent mutations to create a new restriction site for identifying positive worms by PCR. **Automated CRISPR planner for insertions** – this CRISPR tool provide the user with all possible RNA guides and repair templates for introducing an insertion (e.g. EGFP) into specific locations in the worms' genes, including at the start and end of the gene. Both CRISPR tools design of the repair template is based on the CRISPR method described by Paix A. and Seydoux G. in 2017.

Although the WormCoolKit website was built as a pipeline for modeling human disease in worms, each tool can be used separately in support of any relevant query. The use of the website tools is available to all, no username or password required, and is completely anonymous. The website does not collect or record any information about the users' queries.

In summary, WormCoolKit is a free webtool serving the *C. elegans* community that will greatly help researchers test for gene and amino acid conservation with human genes and/or plan their CRISPR experiments.

573A **A cap-associated ribonucleoprotein complex as a platform for assembly of the *trans*-spliceosome in *C. elegans*** Jonathan Pettitt[1], Peter Eijers[1], Mohammed Al-Khafaji[1], Rotimi Fasimoye[2], Rosie Spencer[1], Marius Wenzel[1], Berndt Müller[11]University of Aberdeen, [2]MRC Protein Phosphorylation and Ubiquitylation Unit Sir James Black Centre School of Life Sciences, University of Dundee

Many eukaryotes modify the activity of their spliceosome, allowing them to replace the nascent 5' UTRs of their pre-mRNAs with a short 'spliced leader'. This exon-like element is derived from a longer precursor RNA (the SL RNA) by a *trans*-splicing reaction. However, the molecular mechanism that enables assembly of the "*trans*-spliceosome" is not known for any organism. Building on earlier foundational work, we have shown that a set of nematode-specific proteins and non-coding RNAs have critical roles in this process. We have recently defined two distinct ribonucleoprotein (snRNP) complexes, which are essential for SL1 *trans*-splicing in *C. elegans*. One snRNP forms around the SL1 RNA, and the other associates with the enigmatic, non-coding SmY RNAs. The SL1 snRNP obviously participates in the *trans*-splicing reaction, but the function of the SmY snRNP was previously unclear. To shed light on the function of this latter complex, we performed RIP-Seq using two of its protein components, SNA-3 and SUT-1. We found that these proteins associate with the 5' UTRs of most, if not all, nascent capped RNA polymerase II transcripts, with

the distribution of mapped reads indicating that they are closely associated with the 5' cap. We have previously shown that these proteins interact with the CBC-ARS2 complex[‡], so it is likely that this is how they are recruited to the pre-mRNA. We propose that recruitment of the SmY RNP to the 5' ends of nascent pre-mRNAs is a novel signal that recruits the SL1 RNP, initiating the assembly of the spliceosome and subsequent spliced leader *trans*-splicing of the target mRNA.

[‡] Fasimoye et al. (2022) A novel, essential *trans*-splicing protein connects the nematode SL1 snRNP to the CBC-ARS2 complex. Nucleic Acids Res. 50: 7591–7607.

574A **RNAi-mediated regulation of *alg-3* and *alg-4* coordinates the spermatogenesis developmental program in *C. elegans*** Cara McCormick, Alicia K. Rogers Department of Biology, University of Texas Arlington

The coordination of gene regulatory networks during development is necessary for maintaining fertility. In *C. elegans*, the totipotent germ cells can develop into oocytes or sperm, thus inappropriate activation or silencing of genes responsible for promoting either fate can have detrimental effects on an organism's reproductive potential. Spermatogenesis and oogenesis are temporally separated within the germline tissue during the L4 and adult developmental stages, respectively. Yet, it remains unclear how these developmental programs are robustly executed, particularly during stressful conditions. Here we show RNA interference (RNAi) pathways act to restrict expression of spermatogenesis genes to the L4 developmental stage during heat stress. RNAi pathways use Argonaute proteins complexed with small RNAs to transcriptionally and post-transcriptionally regulate genes and plays key roles in development and fertility. We performed differential expression analysis of mRNA-seq and small RNA-seq libraries from L4 and adult-stage wild-type and *mut-16* mutants, which lack a critical RNAi pathway protein, grown at permissive (20ºC) and elevated temperature (25ºC). Our analyses revealed spermatogenesis-enriched gene expression is developmentally mis-regulated in a small RNA-dependent manner at elevated temperature. In heat stressed *mut-16* mutants, spermatogenesis genes are silenced during the L4 stage, when spermatogenesis typically occurs, and then activated during the adult stage, when oogenesis occurs. Previously, it was shown that the ALG-3/4 branch of the RNAi pathways regulates expression of spermatogenesis genes and is critical for thermotolerant male fertility. Disruption of the ALG-3/4 pathway at elevated temperature leads to failure of spermatids to activate into mature sperm during spermiogenesis. We found that the genomic loci of *alg-3* and *alg-4* are targeted by small RNAs, and that during heat stress, MUT-16-dependent small RNAs are required for L4 stage-specific expression of the Argonautes, ALG-3 and ALG-4. In addition, a sperm activation assay revealed that, like ALG-3/4 pathway mutants, spermatids of heat stressed *mut-16* mutants fail to properly activate. These findings indicate that RNAi pathways are essential for properly coordinating the developmental program of spermatogenesis during heat stress. We propose that appropriate expression of spermatogenesis genes is achieved through small RNA-mediated genetic switches that regulate the expression of ALG-3 and ALG-4 to control ALG-3/4 pathway function throughout development. Moreover, this work provides key insights into the different molecular mechanisms that RNAi pathways employ to maintain both maternal and paternal germ cells' reproductive potential, and further highlights the complexities and importance of RNAi-mediated gene regulation in development.

575A **Identification and Characterization of New Regulators of SKN-1/Nrf** Danielle A Garsin, Carolaing Gabaldon, Larissa Tavizon, Arles Urrutia Microbiology and Molecular Genetics, The University of Texas HSC Houston

Caenorhabditis elegans contains a functional ortholog of the mammalian Nrf proteins called SKN-1, which is a transcription factor that upregulates protective responses and has roles in development, oxidative stress resistance, and lifespan. Like Nrf2, SKN-1 additionally provides resistance to pathogens, including the human bacterial pathogens *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Reactive oxygen species (ROS) generated in response to intestinal infection by these pathogens induces SKN-1 activation in part by activating the p38 mitogen activated protein (MAP) kinase cascade. Many SKN-1 regulators were previously identified by screens, including screens under non-stress conditions, and following exposure to chemical oxidants. Our lab screened on pathogen for SKN-1 regulators and have found additional genes whose loss inhibits SKN-1 activation. We are currently studying genes that encode a F-box protein, components of the V-ATPase, and factors whose loss increases ER stress signaling. The genes identified and hypotheses for their mechanisms of action in affecting SKN-1 activation will be discussed in this presentation.

576A **Context-dependent transcriptional responses to BMP signaling in *C. elegans*** Mehul Vora[1,2], Jonathan Dietz[1], Zachary Wing[3], Jun Liu[4], Christopher Rongo[1], Cathy Savage-Dunn[51]Waksman Institute, Rutgers Unviersity, [2]ModOmics, [3]Queens College, CUNY, [4]Cornell University, [5]Queens College and the Graduate Center, CUNY

Smads and their transcription factor partners mediate the transcriptional responses of target cells to secreted ligands of the TGF-β family, including those of the conserved bone morphogenetic protein (BMP) family. A relatively small number of direct target genes of Smads have been well characterized since the discovery of Smads more than 25 years ago. The current availability of genome-wide "omics" techniques permits questions about Smad transcriptional activity to be addressed on a broader scale. In *C. elegans,* the BMP 2/4 ortholog DBL-1 regulates multiple biological functions, including body size, fat accumulation,

innate immunity, and mesodermal patterning, via a canonical receptor-Smad signaling cascade. To address how this signaling pathway mediates these functions, we elucidated the transcriptional activities of SMA-3/Smad and its transcriptional partner SMA-9/Schnurri. We adapted the mammalian software BETA for *C. elegans*, allowing us to identify functional binding sites based on ChIP-seq peaks (performed by modEncode) and expression differences of nearby genes identified from RNA-seq analysis. We found that SMA-3 and SMA9 have both overlapping and unique target genes. Using BETA software, we were able to separate regulated genes into direct and indirect target genes. At a genome-wide scale, SMA3/Smad acts primarily as a transcriptional activator. In contrast, SMA-9/Schnurri direct targets include both activated and repressed genes. In a *sma-3;sma-9* double mutant, we find evidence that *sma-9* partially suppresses the small body size phenotype of *sma-3,* suggesting some level of antagonism between these factors. This complexity in the interaction between SMA-3/Smad and SMA9/Schnurri function challenges the simpler model for Schnurri function that currently prevails from work in Drosophila and vertebrates. GO term analyses for SMA-3 and SMA-9 identify overrepresentation of functions in lipid metabolism and immune response. To identify target genes that mediate body size regulation, we have analyzed mutants and RNAi-depleted animals. Our findings reinforce our previous conclusion that components of the cuticle are critical in mediating body size regulation. These analyses thus shed light on the transcriptional regulatory networks that mediate biological functions of BMP signaling in size regulation, lipid metabolism, and innate immunity.

577A **Heritable epigenetic changes are constrained by the dynamics of regulatory architectures** Antony JoseCell Biology and Molecular Genetics, University of Maryland

Interacting molecules create regulatory architectures that can persist despite turnover of individual molecules. Although epigenetic changes occur within the context of such architectures, there is limited understanding of how they influence heritability. Here I define criteria for the heritability of regulatory architectures and use quantitative simulations to analyze how they impact the stability of changes across generations. The information transmitted using regulatory architectures is vast and grows rapidly with the number of interacting molecules. While these architectures can recover after many epigenetic perturbations, some resulting changes can become heritable. Such heritable epigenetic changes can alter steady state levels while preserving the architecture, induce different architectures that persist for many generations, or collapse the entire architecture. Differences in outcomes after permanent genetic perturbations versus transient epigenetic perturbations can be used to distinguish regulatory architectures. More complex architectures permit more heritable epigenetic changes that are accessible through both loss-of-function and gain-of-function perturbations. Architectures within the germline that are by themselves unstable can become heritable through periodic interactions with somatic cells, which suggests that the evolution of mortal somatic lineages that reproducibly interact with the immortal germ lineage could be a mechanism for making a wider variety of regulatory architectures heritable. Together, these results provide a foundation for analyzing the inheritance of epigenetic changes within the context of the regulatory architectures formed by diverse molecules in different living systems. Application of this approach for understanding heritable epigenetic changes associated with small RNA-mediated silencing in *C. elegans* will be discussed at the meeting.

578A **Transcriptome profiling the *Caenorhabditis elegans* intestine reveals how ELT-2 negatively and positively regulates intestinal gene expression within the context of a gene regulatory network** Robert T. P. Williams, David C King, Izabella R Mastroianni, Jessica L Hill, Nicolai W Apenes, Gabriela Ramirez, E. Catherine Miner, Andrew Moore, Karissa Coleman, Ambika Basu, Erin Osborne NishimuraBiochemistry & Molecular Biology, Colorado State University

ELT-2 is a major regulator of *Caenorhabditis elegans* intestinal gene expression. ELT-2 initiates in embryos then persists through larval and adult stages. To better understand the contribution of ELT-2 on intestinal transcription and how that contribution changes over time, we performed transcriptome profiling on isolated *C. elegans* intestinal cells across developmental stages. Previously, transcriptome profiles of intestines were generated either on mixed-stage worms or for individual stages that could not easily be compared. We compared our time course of intestinal transcriptome profiles against stage-matched ELT-2 ChIP-seq data from the modENCODE and modERN projects (van Nostrand, et al. 2013; Kudron et al, 2018) and with transcriptome profiles from worms depleted for *elt-2* (Dineen and Osborne Nishimura et al., 2018). We found that only 33% of intestine-enriched genes in the embryo were direct targets of ELT-2 but that this number increased to 75% by the L3 stage. This suggests additional transcription factors promote intestine-specific transcription especially in the embryo. Furthermore, only half of ELT-2's direct target genes were dependent on ELT-2 for their proper expression levels. Of the target genes that depended on ELT-2, an equal proportion responded to *elt-2* depletion with over-expression as with under-expression. That is, ELT-2 can activate or repress direct target genes. Additionally, we observed that ELT-2 repressed its own promoter, implicating its autoregulation may involve both negative and positive feedback. To identify additional transcription factors that regulate intestine-specific genes we used the modENCODE and modERN ChIP-seq ChIP-seq datasets and found that ELT-2 target genes are typically also occupied by PQM-1, PHA-4, and UNC-86. Overall, this work found that ELT-2 regulates 20 – 50% of intestine-specific genes, both positively and negatively controls its direct targets, and works in the context of a larger gene regulatory network.

To delve into how individual intestinal cells function with greater detail, we developed a protocol for single-cell RNA-seq of isolated and enriched intestinal cells. This assay will contribute to ongoing studies aimed at determine how the intestine sub-func-

tionalizes and responds to different bacterial exposures and diets.

579A **CGC1, a new gap-free and telomere-to-telomere reference genome and isogenic wild-type strain for *Caenorhabditis elegans*** Erich M Schwarz[1], Kazuki Ichikawa[2], Massa J Shoura[3], Karen L Artiles[3], Chie Owa[2], Haruka Kobayashi[2], Manami Kanamori[4], Yu Toyoshima[4], Yuichi Iino[4], Ann E Rougvie[5], Andrew Z Fire[3,6], 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]Dept. of Genetics, Stanford University

HiFi PacBio with ultra-long Oxford Nanopore sequencing has made it possible to achieve gap-free and telomere-to-telomere reference genome assemblies for complex eukaryotes. So far, only three such genomes have been published: human, watermelon, and the nematode *Oscheius tipulae*. After five decades of use, the standard wild type strains used in *C. elegans* laboratories (collectively referred to as «N2») have accumulated genetic polymorphisms leading to observable phenotypic differences between different laboratories. These genetic inconsistencies meant that the N2 reference genome did not exactly correspond with any existing N2 strain. To solve this problem, we generated CGC1 (originally called PD1074), a completely isogenic wild-type derivative of N2 that is cryopreserved in numerous aliquots at the CGC and available to all. To assemble a corresponding gap-free and telomere-to-telomere reference genome from CGC1 (PD1074), we used HiFi PacBio and ultra-long Nanopore to assemble 61 contigs with hiCanu, order them chromosomally via a preexisting reference, and close 11 gaps of <1 kb with the consensuses of Nanopore reads. This left 43 gaps filled with long tandem repeats of ≥10 kb in size that we closed through localized hybrid assembly, first closing the 43 gaps with Nanopore reads and then mapping, aligning, and generating consensuses from HiFi reads. Our resulting CGC1 genome has six nuclear chromosomes totaling 105.9 Mb and a mitochondrial chromosome of 13,994 nt. It encompasses 100.3 Mb of the previous N2 reference assembly, along with an extra 5.6 Mb containing all 12 telomeres, at least 50 newly identified protein-coding genes, and 5.3 Mb of added tandemly repeated sequences, including repeated regions such as pSX1 (153 kb) that span all but the longest Nanopore reads. Structural variations in outgroup *C. elegans* strains indicate that most disagreements between the older N2 and the newer CGC1 assemblies are due to erroneous omissions in N2. The CGC1 reference and its matched isogenic strain will enable maximum precision for future genomic analyses of the model organism *C. elegans*.

580A **DOT-1.1 (DOT1L) deficiency in *C. elegans* leads to small RNA-dependent gene activation** Thomas Liontis[1], Karisma Verma[1], Alla Grishok[21]Boston University School of Medicine, [2]Biochemistry, Boston University School of Medicine

The *C. elegans* DOT-1.1 histone H3 lysine 79 methyltransferase is an ortholog of the mammalian DOT1L protein. Both DOT-1.1 and DOT1L, unlike yeast Dot1, are recruited to chromatin by animal-specific histone-binding factors. In *C. elegans*, these factors are ZFP-1 and GFL-1. Notably, *zfp-1* and *gfl-1* belong to a group of mutants with reduced (but not absent) efficiency of experimental dsRNA-induced gene silencing, RNAi. The mechanism of this phenomenon remained elusive for a long time. Through studying the role of ZFP-1 and DOT-1.1 in transcription regulation we found elevated bi-directional transcription in a *zfp-1* mutant deficient in DOT-1.1 recruitment to chromatin. This prompted us to hypothesize that bi-directional transcripts formed dsRNA recognized by the Dicer complex and that ectopic endogenous dsRNA production prevented efficient utilization of the exogenous dsRNA thus causing reduced RNAi efficiency in *zfp-1* mutant background. First, we tested the idea of elevated RNAi pathway activation genetically. For this, we took advantage of a strong early lethal phenotype of the *dot-1.1* deletion allele deficient in H3K79 methylation. We found that *dot-1.1(-)* lethality was completely suppressed by the *rde-4* and *rde-1* mutants of the canonical dsRNA-responsive RNAi pathway. This suggested that ectopic siRNAs matching some target genes caused *dot-1.1(-)* lethality. Since *dot-1.1(-)* lethality was also suppressed by *ced-3* (caspase) deficiency, we used *ced-3(-)* and *dot-1.1(-); ced-3(-)* mutant embryos for RNA-seq and small RNA-seq experiments. In the latter case, we specifically looked at primary siRNAs containing 5'-monophosphate. We have not observed dramatic perturbations of the small RNA landscapes driven by *dot-1.1(-)* and instead found an elevated abundance of select primary siRNAs. Surprisingly, these elevated siRNAs matched genes upregulated, but not downregulated, by *dot-1.1(-)* with high significance. The siRNA target genes' upregulation by *dot-1.1(-)* was dependent on RDE-4, consistent with the role of siRNAs in gene activation. Moreover, functional analysis of the upregulated genes revealed enrichment in proteolytic function. Also, genes previously reported downregulated in the *alg-5* Argonaute mutant were strongly enriched among those upregulated by *dot-1.1(-)*. Overall, our findings are consistent with a model where RDE-4-dependent siRNAs bind RDE-1 and/or ALG-5 Argonautes to potentiate the expression of proteolysis genes in *dot-1.1(-)* background.

581A **The transgenerational accumulation of repressive H3K9me2 affects health and lifespan in *C. elegans*** Natilia Woozencroft, Jaime C Croft, Arthur Colunga, Lea Solh, Michaela K Dillon, 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 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 health metrics like motility, reversal frequency, pharyngeal pumping, and lipid levels 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.

582A **Rethinking microRNA-mediated regulation of *lin-28* in the *C. elegans* heterochronic network** Charles Nelson, Victor Ambros Molecular Medicine, UMass Chan Medical School

In *C. elegans*, genes that function in the heterochronic regulatory network are necessary for the proper progression of cell fates throughout development. Mutations in heterochronic genes result in precocious or retarded development of certain cell lineages. The *lin-4* microRNA and the *let-7*-family of microRNAs are critical negative regulators of multiple core heterochronic genes, including the L1-promoting gene *lin-14* and the L2-promoting genes *hbl-1* and *lin-28*, whose protein levels negatively correlate with expression of *lin-4* and *let-7*-family microRNAs.

Previous studies have shown that both the *lin-14* and *hbl-1* 3' UTRs contain multiple sequences (sites) complementary to *lin-4* and *let-7*-family microRNAs, and that deletion of their respective 3' UTRs results in 1) misexpression of LIN-14 and HBL-1 proteins at later larval stages, and 2) extremely penetrant gain-of-function (retarded) phenotypes much like the loss of *lin-4* and *let-7*-family microRNAs. To date, no study has investigated the regulatory functions of the endogenous *lin-28* 3' UTR. Here, we explore the roles the *lin-28* 3' UTR and its *lin-4* and *let-7*-family microRNA sites in regulating the developmental dynamics of LIN-28 protein.

We find that deletion of the *lin-4* and *let-7*-family sites in the endogenous *lin-28* 3' UTR results in a highly penetrant gain-of-function (retarded) phenotype. Surprisingly, deletion of the entire *lin-28* 3' UTR results in mild heterochronic phenotypes. These results indicate that, unlike the *lin-14* or *hbl*-1 3' UTRs, the *lin-28* 3' UTR contains strong positive regulatory elements that appear to balance the negative regulation by *lin-4* and *let-7*-family microRNAs, and that the LIN-28 protein can be downregulated in the absence of its 3' UTR. Through the generation of multiple *lin-28* 3' UTR truncations, positive regulatory elements have been identified in at least three regions, and efforts are currently underway to characterize the nature of these elements.

During our analyses of the *lin-28* 3' UTR, we have discovered that endogenously tagging either end of the LIN-28 protein can result in suppression of the gain-of-function phenotypes observed when the *lin-4* and *let-7* microRNA sites are deleted, whereas internal tagging of LIN-28 does not result in suppression. These results suggest that end tagging of LIN-28 decouples the modes of regulation imposed on *lin-28* by its 3' UTR and underscores the practical matter of the need for caution when using tags to study protein function.

583A **Formation of fountains by cohesin in nematodes: micro-TADs for the limitation of enhancer search space** Bolaji Isiaka[1], Anja Haemmerli[1], Jennifer Semple[1], Moushumi Das[1], Valeriia Volodkina[1], Daniel Jost[2], Peter Meister[1]1University of Bern, [2]ENS Lyon

In most species that have been studied, chromosomes are divided into 3D compartments known as topologically associated domains (TADs). The creation of TADs involves the interplay between cohesin, which is a member of the structural maintenance of chromosome (SMC) complexes family, and boundary sequence elements recognized by transcription factors. TADs are highly conserved between cell types and between species in syntenic regions. They play a critical for transcriptional regulation by limiting the search space of enhancers for target promoters.

While TADs are present in most species, chromosome conformation capture studies using entire animals have failed to detect them in worms, except on the dosage-compensated X chromosome. However, tens of thousands of sequences with chromatin features characteristic of enhancers have been identified. This raises the question of how enhancer-promoter specificity is directed and how enhancer activity is limited.

Using high-resolution genome-wide chromatin conformation capture, we discovered that enhancer sequences are correlated

with 3D hairpin structures extending 10-50 kb from the enhancers, which we call “fountains”. Fountains are specific to active enhancers, accumulate the major somatic cohesin, and disappear when the latter is cleaved *in vivo*. Additionally, fountains accumulate topological constraints, bind topoisomerases and the negatively supercoiled DNA binder psoralen. Functionally, fountain disappearance correlates with enhancer-proximal gene activation, suggesting that fountains play a role similar to TADs in directing enhancer-promoter interactions in *C. elegans*.

584A **The role of the endogenous nuclear RNAi pathway in chromatin remodeling during *Caenorhabditis elegans* dosage compensation** Hector Mendoza, Michael B Davis, Sarah VanDiepenbos, Rebecca A Haines, Györgyi Csankovszki Molecular, Cellular and Developmental Biology, University of Michigan

A landmark of dosage compensation in *Caenorhabditis elegans* involves X chromosome compaction and sequestration at the nuclear lamina. This process reinforces dampening of gene expression from both hermaphrodite X chromosomes to maintain gene dosage balance between the biological sexes. We recently demonstrated that disrupting the endogenous nuclear RNAi pathway leads to increased levels of X chromosome decondensation and less effective dosage compensation. In the endogenous RNAi pathway, the worm-specific Argonaute proteins HRDE-1 (germline targets) and NRDE-3 (somatic targets) recruit the histone methyltransferases MET-2, SET-25 and SET-32, which either directly or indirectly contribute to the deposition of H3K9 trimethylation marks. These methyltransferases are also known to contribute to X chromosome condensation and dosage compensation. We used immunofluorescence microscopy to examine the level of X chromosome decondensation when the RNAi machinery and the histone methyltransferases are simultaneously disrupted. Our results suggest that NRDE-3 impacts X chromosome condensation via the recruitment of the methyltransferases, while HRDE-1›s involvement is through an additional pathway different from MET-2, SET-25 and SET-32. We also assess the effects on dosage compensation at the level of transcription using RNA-seq. Our findings expound how the RNAi machinery contributes to changes in chromatin architecture to influence global gene expression of the hermaphrodite X chromosomes during the dosage compensation mechanism.

585A **The role of chromatin-interacting replisome subunits in transgenerational epigenetic inheritance (TEI)** Juan Carlos Rueda Silva[1,2], Giulia Furlan[1], Lisa Lampersberger[1,2], Eric Miska[21]Department of Genetics, University of Cambridge, [2]Department of Biochemistry, University of Cambridge

Transgenerational epigenetic inheritance (TEI) the process through which changes in gene expression are maintained across multiple generations, in absence of the initial trigger in a mutation-independent manner. In *Caenorhabditis elegans*, RNA interference (RNAi) is a trigger of TEI via the nuclear RNAi pathway, which results in both the synthesis of 22G-RNAs and the deposition of repressive chromatin marks in the targeted loci. The inherited signal remains elusive.

Previous research has shown the importance of replisome-chromatin interactions in the maintenance of gene repression. The DNA helicase subunit MCM-2 is crucial for the distribution of parental H3/H4 histone dimers between the leading and lagging strands, the leading strand DNA Polε subunits POLE-3 and POLE-4 are involved in the maintenance of parental H3/H4 dimers, and the DNA polα subunit POLA-1 plays a role in the inheritance of parental H2A/H2B dimers.

We have generated several mutant *C. elegans* strains. These strains had non-functional histone binding domains of MCM-2, POLA-1, POLE-3 or POLE-4. Therefore, they exhibit impaired maintenance of parental histone marks. As such, these strains exhibit phenotypes commonly associated with improper chromatin marking, such as showing germline mortal (Mrt), high incidence of males (Him), bursting vulva and dumpy-like phenotypes. In addition, these mutations are correlated with a faster loss RNAi-induced silencing memory. Suggesting the role of chromatin-replisome interaction in the transgenerational inheritance of RNAi-induced gene silencing.

586A **Last(?) piece of the puzzle: piRNA processing by a trimeric Schlafen-domain nuclease** Nadezda Podvalnaya[1], Alfred Bronkhorst[1], Raffael Lichtenberger[2], Svenja Hellmann[1], Emily Nischwitz[1], Torben Falk[2], Emil Karaulanov[1], Falk Butter[1], Sebastian Falk[2], Rene Ketting[11]Institute of Molecular Biology, [2]Max Perutz Labs, University of Vienna

Transposable elements are genomic parasites that expand within and spread between genomes. In the germline, the activity of such elements is controlled, amongst others, by Piwi proteins. These proteins recognize their targets through small RNA co-factors named piRNAs, making piRNA biogenesis a key specificity-determining step in this crucial genome immunity system. While the processing of piRNA precursors is an essential step in this process, many molecular details of this process remain unknown. We identify a novel endoribonuclease complex, PUCH, that initiates piRNA processing in *Caenorhabditis elegans*. Genetic, biochemical and modelling studies show that PUCH, a trimer of Schlafen-like-domain proteins (SLFL proteins), executes 5’-end piRNA precursor cleavage. Interestingly, PUCH-mediated processing strictly requires an m7G-Cap and a uracil at position three (i.e. the position that will become the 5’-end of the novel piRNA. We also demonstrate, through biochemical and structural studies, how PUCH interacts with PETISCO, a complex that we previously showed to be required for efficient piRNA biogenesis and to bind piRNA precursors. We also validated that this interaction enhances piRNA production *in vivo*. The identification of PUCH

completes the repertoire of *C. elegans* piRNA biogenesis factors and uncovers a completely novel type of RNA endonuclease, formed by three SLFL proteins. Mammalian Schlafen (Slfn) genes have been associated with immunity responses, exposing a thus far unknown molecular link between immune responses in mammals and deeply conserved RNA-based mechanisms that control transposable elements.

587A **SmY RNAs, an essential family of nematode-specific non-coding RNAs that play a key role in spliced leader *trans*-splicing** Mohammed Alkhafaji[1], Berndt Muller[1], Jonathan Pettitt[1], Rotimi Fasimoye[21]University of Aberdeen, [2]University of Dundee

Spliced Leader *trans*-splicing exists in many eukaryotic groups. This post-transcriptional process involves replacement of the 5' UTRs of pre-mRNAs with a "spliced leader" sequence provided by a longer precursor called the SL RNA. In *C. elegans*, two ribonucleoprotein complexes are involved in SL1 *trans*-splicing: the SL1 RNP and the SmY RNP that contains the enigmatic SmY RNAs. Unlike the SL1 RNP, SmY RNPs do not participate directly in the *trans*-splicing reaction and their function was previously unknown. The first SmY RNA was discovered in spliceosomal extracts of *Ascaris lumbricoides* and subsequent work showed this to be the founding member of nematode-specific family of short non-coding RNAs.

C. elegans has 12 *smy* genes, each encoding a distinct SmY RNA. To systematically investigate SmY function, we knocked out all 12 genes. Molecular assays showed that animals lacking ten of the twelve *smy* genes have defects in spliced leader *trans*-splicing but are nonetheless viable, while loss of 11 *smy* genes reduces animal viability by 80%. Removal of all 12 genes results in animals that arrest during larval development, characteristic of the phenotype we see in other SL *trans*-splicing component mutants. These data show that SmY RNAs are required for spliced leader *trans*-splicing.

We are currently using transgenic rescue assays to dissect the molecular determinants of SmY RNA function. We have shown that one wild type SmY RNA "SmY-10" is non-functional, although it is expressed and able to interact with Sm proteins.

As part of work to better define the molecular partners of the SmY RNAs, we have shown that SmY RNAs tagged with the MS2 stem-loop (a motif that recognizes the coat protein from the MS2 bacteriophage) are functional. We have used this MS2-tagging approach to confirm that SmY RNAs are nuclear. We are currently generating strains for SmY RNA immunoprecipitation, and also examining the role of the SmY RNAs on the nuclear localisation of spliced leader *trans*-splicing components.

588A **Functional characterization of GTSF-1 across clade V nematodes reveal conserved role in RNA-Dependent RNA polymerase activity as well as novel interactions with PETISCO complex** Shamitha Govind[1], Emily Nischwitz[1], Svenja Hellmann[1], Ann-Sophie Seistrup[1], Hahn Witte[2], Ralf Sommer[2], Falk Butter[1], Rene Ketting[11]Institute of Molecular Biology, [2]Max Planck Institute for Biology Tübingen

Small-RNAs (sRNAs) regulate gene expression by binding to an Argonaute (Ago) protein and interfering with mRNA transcription or translation. One conserved component of animal sRNA pathways is the Zn finger protein Gametocyte specific factor -1 (GTSF-1). In *D. melanogaster*, *M. musculus* and *B. mori,* GTSF-1 assists the PIWI Ago to silence germline transposons through Piwi-interacting small RNAs (piRNAs). However, in *C. elegans,* GTSF-1 binds the RNA Dependent RNA Polymerase RRF-3, and enables the RRF-3 driven biogenesis of 26G RNAs. These sRNAs regulate protein-coding genes, pseudogenes and duplicated genes in *C. elegans* germline.

RdRPs are generally absent from most animals but are widely conserved across nematodes. We therefore wanted to probe how conserved the identified function of GTSF-1 is within nematodes. To address this, we are characterizing GTSF-1 in clade V nematodes *C. briggsae* and *P. pacificus* using transgenic techniques, transcriptomics and proteomics.

With a predictive software, we show that nematode GTSF-1 proteins have unique structural differences such as loss of a positively charged stretch that enables RNA binding in *M. musculus* and *D. melanogaster* GTSF-1. In *C. elegans*, loss of GTSF-1 leads to a loss of 26 RNAs due to which the animals have severe defects in fertility. We expressed *C. briggsae* GTSF-1 in *C. elegans* and found it to rescue both fertility defects and 26G RNA levels. We then show that deletion of *gtsf-1* from *C. briggsae* and *P. pacificus* also lead to defects in fertility and loss of 26G RNAs. Finally, we use yeast-two-hybrid and IP-MS to show that both *C. briggsae* and *P. pacificus* GTSF-1 interact with RRF-3 homologs. Thus, the molecular function of GTSF-1 that we identified in *C. elegans* appears to be conserved across clade V nematodes.

Interestingly, our MS data also showed that *C. briggsae* GTSF-1 associates with another complex called PETISCO; this complex is known to interact with precursor transcripts of piRNAs but had not been linked to 26G RNAs. A closer look at our data from various PETISCO mutants in *C. elegans* indeed revealed a small but significant loss of 26G RNAs. We hypothesize that in both *C. briggsae* and *C. elegans*, PETISCO has an additional role to recognize transcripts from which 26G RNAs are synthesized. We are now validating these findings using CRISPR mutants, yeast-two hybrid and iCLIP assays.

In summary, our study highlights the structural and functional differences in nematode GTSF-1 compared to other animal homologs. The link between PETISCO and GTSF-1 also demonstrates the evolution of small-RNA pathways from existing cellular machinery and sheds light on the flexible design of molecular pathways.

589A **Elucidating the molecular and genetic mechanisms of action of cocaine by leveraging *C. elegans* genetics and behavior** Emily Williams[1], Rachid El Bejjani[21]Davidson College, [2]Biology, Davidson College

Pharmacological, biochemical, and behavioral studies have shed significant light on some of the main molecular mechanisms of cocaine associated with drug seeking behavior. However, inhibition of these pathways alone is not sufficient to treat cocaine addiction, suggesting that additional mechanisms that remain unknown are involved. The fast generation time, paired with the huge number of genetic resources available, and with imaging and behavior tractability make *C. elegans* a very attractive model system to accelerate the process of discovery in the elucidation of the cellular and molecular mechanisms triggered by cocaine. In our recent paper, we showed that Acetylcholine release is required for cocaine dependent egg laying in *C. elegans*. More recently, we have shown that some of the effects of cocaine are enhanced in *mod-1* mutants in a serotonin-dependent manner, suggesting a complex mechanism involving at least two neurotransmitter systems. To follow up on our findings, we asked how acute exposure to cocaine affects gene expression in Acetylcholine neurons and in other neuron populations. We treated animals with cocaine and performed cellular dissociations and FACS sorting followed by RNA Seq analysis of the sorted neurons in cocaine treated and control animals. We identified some genes that are differentially expressed in a similar fashion in all neurons and others that are differentially expressed in Ach neurons specifically or only in neurons other than Ach neurons. The majority of differentially expressed genes are upregulated in cocaine treated worms. Clusters of differentially expressed genes include signaling enzymes, ion channels, neuropeptides and their receptors, transcription factors, as well as genes known to affect neuronal differentiation, axon guidance, synaptogenesis, and other neurodevelopmental mechanisms. We are currently following up on these results using genetic analysis and behavioral methods in *C. elegans*.

590A **From WormBase to the Alliance of Genome Resources – Developments and Data Migrations** Stavros Diamantakis[1], Andres Becerra Sandoval[1], Manuel Luypaert[1], Mark Quinton-Tulloch[1], Paul Sternberg[2], Sarah C Dyer[11]EMBL-EBI, [2]California Institute of Technology

WormBase (www.wormbase.org) is the central repository for information concerning the genetics, genomics and biology of *Caenorhabditis elegans.* WormBase is one of the founding members of the Alliance of Genome Resources (www.alliancegenome.org), which also brings together data from other model organism databases (Drosophila, mouse, rat, yeast, Xenopus and zebrafish) to provide harmonised views of these data with an emphasis on comparative genomics to help understand the genetic and genomic basis of human biology, health and disease.

From the Alliance site you can access a range of WormBase data for *C. elegans* including gene models, Gene Ontology annotations, alleles/variants and disease curations. Gene pages display orthologues from the other model species and human, functional and pathway information, strain details, expression data by anatomical structure / developmental stage, and molecular and genetic interactions. Additional tools and datasets will continue to be added to Alliance, and there are links between Alliance and WormBase pages to help users take advantage of the full functionality of both resources.

591A **Chromatin modifier SET-25/G9a is required to modulate the transcriptome revealing different adult gene expression and behavioral profile in the next generation after early life stress in Caenorhabditis elegans** Aina Bellver Sanchis[1], David Valle-Garcia[2], Eszter Gecse[3], Mercè Pallàs[1], Csaba Söti[3], Christian Griñán Ferré[11]Universitat de Barcelona, [2]Institute of Biotechnology, National Autonomous University of Mexico, [3]Semmelweis University

Early-life stress experiences form permanently imprinted memories that can persistently alter expression levels of key genes leading to changes in behavior, molecular response, and stress throughout later life, including subsequent generations. While transgenerational epigenetic inheritance has been studied, there are still some gaps in our knowledge. Our experimental paradigm demonstrated that the Caenorhabditis elegans (*C. elegans*) form an imprinted behavioural and cellular defence memory in response to early-life stresses. We exposed newly-born worms (eggs) to toxic antimycin (AM) which promotes aversive behavior through chemotaxis assays and stimulates *hsp-6:GFP* expression, a toxin-specific cytoprotector. Learned adult defenses require memory formation during the L1 larval stage and do not appear to confer increased protection against the toxin. In our study, aversive behavior was inherited only to the F1 generation after one exposure to toxins, but molecular alterations were observed up to F5 generation. Changes in the gene expression of the chromatin modifier *set-25/G9a* were observed, as well as the gene expression of *hsp-6*, *skn-1*, *gst-4* and *atfs-1* after 1 exposure to the toxic. Besides, we found differences in lifespan after 1 exposure up to F3 generations. Moreover, in our RNA-sequencing analysis, we found that the expression of 2.299 genes changes in any of the generations with OP50 treatment. Many of these changes related to aging and neurodegenerative disorders were observed in F1, and the gene expression of most of them was decreased compared to the control group. Interestingly, among

other epigenetic-related enzymes, we found an increase in the gene expression of *set-28*, a histone methyltransferase from the SET domain superfamily, after exposure to AM in the F1. The results of this study support the hypothesis that chromatin modifiers play a crucial role in the establishment of transgenerational epigenetic inheritance (TEI) memory and modulate pathways associated with behavior and aging. In this way, toxic stress during the critical period can lead to adaptive behavioral and cytoprotective responses, as well as enhancing health outcomes, showing a wide range of outcomes that can result from an early-life stressful experience. Thus, it opens a new pathway for understanding how methyltransferases function during early life stress during TEI.

592B **A neural-specific mechanism to regulate PQM-1 expression and survival from hypoxia** Ananya Mahapatra, Alfa Dhakal, Aika Noguchi, Pranathi Vadlamani, Heather A Hundley Indiana University

The transcription factor PQM-1 is an important mediator of the insulin signaling pathway contributing to longevity and the stress response as well as promoting survival from hypoxia. Herein, we reveal a novel mechanism for regulating PQM-1 expression specifically in neural cells of hatched L1 animals. Our studies reveal that the RNA binding protein, ADR-1, binds to *pqm-1* mRNA in neural cells and reduces expression of both *pqm-1* and downstream PQM-1 activated genes. ADR-1 binding to *pqm-1* is regulated by the presence of a second RNA binding protein, ADR-2, which heterodimerizes with ADR-1. Interestingly, we find that neural *pqm-1* expression is sufficient to both impact gene expression throughout the animal and affect resistance to survival from hypoxia; phenotypes which are also observed in *adr* mutant animals. Together, these data indicate an important post-transcriptional gene regulatory mechanism that allows the nervous system to sense and respond to environmental conditions to promote organismal survival from hypoxia.

593B **Using TWIST1 patient mutations in HLH-8 structure/function studies** Michael J. Gruss, Colleen O›Callaghan, Molly Donnellan, Ann K Corsi Biology, The Catholic University of America

TWIST1 is a basic helix-loop-helix (bHLH) transcription factor in humans that regulates genes that are important for cell differentiation. Autosomal dominant mutations of TWIST1 lead to haploinsufficiency and cause skull developmental defects that are characteristic of Saethre-Chotzen syndrome (SCS). The single TWIST1 ortholog in *C. elegans*, HLH-8, is required for the differentiation of egg-laying and enteric muscles. Null alleles of *hlh-8,* therefore, lead to egg-laying defective (Egl) and constipated (Con) phenotypes due to defective muscle development. TWIST1 and HLH-8 share the highest amino acid identity in their bHLH domains that are responsible for DNA binding and dimerization. Another domain of TWIST1, called the TWIST-Box, has been shown to be important for the protein's transcriptional activity. HLH-8 has a region of low amino acid identity (24%) when compared to the TWIST-Box overall but contains nine amino acids with three equally-spaced residues (L95, F99, R103) that are highly-conserved across many species. Protein modeling predicts these three amino acids reside on the same side of an alpha helix where they may be playing an important role in intermolecular interactions, and we hypothesized would be important for HLH-8's transcriptional activity. To test this hypothesis, we used CRISPR/Cas9 editing to delete the nine amino acids in HLH-8 and were surprised to find milder defects compared to null mutants since the animals could still lay eggs and were not Con. To further investigate this region, we made alanine substitution mutations in the genome at each position (L95A, F99A, R103A) and missense mutations that are analogous to SCS patient mutations (F99L and R103M). We characterized the phenotypes in each of these mutants and examined the expression of two HLH-8 target gene GFP reporters (*arg-1::*GFP and *egl-15*::GFP). The mutants showed tissue-specific, allele-specific, and target gene-specific phenotypes. Further, we found that the conserved amino acids in the HLH-8 TWIST-Box do not contribute equally to the domain's function and that the patient alleles generally had milder defects compared to the alanine substitutions. Additional protein modeling suggested that the patient alleles maintain some of their intermolecular contacts, which could provide a mechanism for the mild patient phenotypes.

594B **An insulin signaling pathway in the parasitic nematode *Brugia malayi*** Kirsten Crossgrove, Cole Lindwall, Alexandra Kestol, William Morgan, India Fleming, Miles Hagen, Jake Minx, Sarah Ferguson Biology, University of Wisconsin-Whitewater

The parasitic nematode *Brugia malayi* is transmitted by mosquitoes and causes lymphatic filariasis in humans. According to the dauer hypothesis, the infective stage (iL3) parasites, which are transmitted 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 in a non-feeding quiescent state until a change in environmental conditions, which then triggers molting to the L4 stage. For dauer, that change is an improvement in environmental conditions (presence of food, less crowding, 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 are using bioinformatic tools and RT-PCR to identify and compare predicted gene structures of the *Bma-daf-2*, *Bma-age-1, Bma-aap-1, Bma-pdk-1, Bma-pptr-1, Bma-akt-1* and *Bma-daf-16* genes, as well as several genes encoding insulin like peptides. We will report on the current status of research on these *B. malayi* genes. Our goal is to use the *B. malayi* genes in transgenic rescue experiments in *C. elegans*. Since the genes in the IIS pathway are highly conserved, we hypothesize that the *B. malayi* orthologs will be able to rescue function

in *C. elegans* mutants, which may then provide a system to further study *B. malayi* protein function.

595B **Characterization of NHR-25 genome-wide binding reveals role for combinatorial transcription factor action** Deborah M Thurtle-Schmidt, Alex L Sinks, Leah Flautt, Kimberley T Muchenje Biology, Davidson College

Terminal cellular differentiation and maintenance into distinct cell types is due in part to sequence-specific transcription factors (TFs) directing proper transcriptional regulation. TFs bind to response elements, establishing the proper gene regulatory network for that cell type. Thus, binding site recognition and subsequent regulation of the TF target gene to establish and maintain the correct gene regulatory network is critical for proper genomic functioning. However, how a TF recognizes its binding site and subsequent target genes remains poorly understood. *C. elegans* provides an excellent model to study transcriptional regulation due to its compact genome and highly conserved TFs. To investigate how a transcription factor identifies its binding site to target the correct target gene we profiled genome-wide binding of an endogenously tagged, highly conserved TF, NHR-25, using ChIP-seq and CUT&RUN in L1 worms. We identified 1140 NHR-25 enriched sites. 78% of these sites overlapped with previous NHR-25 ChIP-seq of a multi-copy integrant NHR-25 from the modERN resource, however the multi-copy integrant resulted in 5x as many enrichment sites, suggesting TF expression level is critical for binding. Motif analysis of NHR-25 occupied regions identified 23 enriched motifs, including the presumed NHR-25 binding site and a GAGA motif like the EOR-1 binding motif. Correlation with *C. elegans* chromatin domains (Evans et al 2016) showed enriched regions containing the NHR-25 motif at regions designated as enhancer-type chromatin. To identify the NHR-25 responsive genes and correlate target genes to NHR-25 enriched regulatory elements, we performed RNA-seq of *nhr-25* knockdown worms and correlated differentially regulated genes to the nearest enriched peak. Many peaks were proximal to, but not directly adjacent to a differentially expressed gene. To directly correlate target genes of identified regulatory elements, we deleted NHR-25 enriched regions using CRISPR/Cas9 and profiled gene expression by RNA-seq. For a single deleted regulatory element, we identified two nearby genes with altered expression. However, only one of these adjacent genes showed NHR-25 dependent expression. Taken together, these data suggest combinatorial action of multiple TFs are required to direct proper gene regulation.

596B **SIN3 acts in distinct complexes to regulate the germline transcriptional program in *C. elegans*** Matthieu Caron[1], Valerie Robert[1], Annie Adrait[2], Victoria Palulska[2], Yohann Coute[2], Manon Chevalier[3], Christian Riedel[3], Cecile Bedet[1], Francesca Palladino[41]LBMC, ENS-Lyon, Lyon University, [2]CEA Grenoble, [3]Karolinska Institute, [4]LBMC, Ecole Normale Supérieure de Lyon, Lyon University

The SIN3 transcriptional coregulator influences gene expression through multiple interactions that include histone deacetylases (HDACs). Haploinsufficiency and mutations in SIN3 are the underlying cause of Witteveen-Kolk syndrome and related intellectual disability (ID)/autism syndromes, emphasizing its key role in development. However, little is known about the diversity of its interactions and functions in developmental processes. Here we show that loss of SIN-3, the single SIN3 homologue in *Caenorhabditis elegans*, results in maternal effect sterility associated with deregulation of the germline transcriptome, including desilencing of X-linked genes. We identify at least two distinct SIN3 complexes containing specific HDACs, and show that they differentially contribute to fertility. Single cell smFISH reveals that in *sin-3* mutants, the X chromosome becomes re-expressed prematurely and in a stochastic manner in individual germ cells. Furthermore, we identify histone residues whose acetylation increases in the absence of SIN3. Together, this work provides a powerful framework for the *in vivo* study of SIN3 and associated proteins.

597B **SET-domain proteins in epigenetic inheritance: hidden depths** Dhruv Monteiro[1], Natasha Jones[1], Joel Mackay[2], Alyson Ashe[11]School of Life and Environmental Science, University of Sydney, [2]University of Sydney

Over the last two decades it has become clear that epigenetic modifications acquired by an individual during its lifetime can be inherited for multiple generations. There are a growing number of examples where there is inheritance from parent to offspring of environmentally acquired gene expression changes. To study this, we have developed a transgenerational epigenetic inheritance (TEI) sensor in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations

Using this system, we found that two putative histone methyltransferases, *set-9* and *set-26*, are involved TEI. These two genes are 98% identical, and are so named because they contain SET domains, domains that usually have lysine methyltransferase activity. Intriguingly, the SET domains of these two proteins contain an unusual catalytic site and their ability to act as histone lysine methyltransferases is controversial. The unusual catalytic site is maintained in the putative human homolog (MLL5), suggesting some functionality. We performed TEI assays in strains containing a one amino acid mutation in the putative catalytic site of the methyltransferase domain and showed that this domain is required for TEI, confirming that it is indeed functional.

SET-9/26 also contain PHD finger domains, thought to bind H3K4me3. We mutated the binding pocket of the PHD fingers and showed that mutant animals have a defect in TEI, confirming that the action of both SET and PHD finger domains is important in epigenetic inheritance. Using NMR, we observe that the affinity of the PHD fingers for H3K4me3 *in vivo* reduces when H3K9 post-translational modifications are also present.

Interestingly, SET-9/26 also have extensive intrinsically disordered regions (IDRs). We discovered another gene, *Y73B3A.1* that contains no structured regions, but is homologous to the IDRs of *set-9/26*. We deleted *Y73B3A.1* and showed that null mutants are also defective in epigenetic inheritance to the same degree as *set-9/26* mutants. These data implicate the IDRs of all three proteins in TEI.

Taken together these data imply a model whereby SET-9 and SET-26 bind histones using their SET and PHD finger domains, suggesting that SET-9/26 may act at the interface between silenced and active chromatin, helping to convert active euchromatin (H3K4me3) to silenced heterochromatin (H3K9me3). The IDRs of all three proteins are also involved, and potentially mediate protein-protein interactions or phase separation.

598B **_De Novo_ designed protein switches in _C. elegans_** Adam P Berg[1], Michael Bertram[2], Henry Giesel[2], Jakob Faber[2], Austin Johnson[2], Joseph Kaefer[2], Mitchell Keeling[2], Mawui Nevis[2], Connor Wakefield[2], Walter R.P. Novak[3], Erika B. Sorensen[41]Biolgy, Wabash College, [2]Wabash College, [3]Chemistry, Wabash College, [4]Biology, Wabash College

The ability to inactivate gene function *in vivo* is essential for understanding the molecular mechanisms that regulate normal and disease phenotypes. RNAi and gene editing techniques have limitations, including 1) slow protein turnover can prohibit use of RNAi; 2) genetic deletion of essential genes prevents characterization of later functions. Recent work to develop methods to conditionally deplete proteins (e.g. AID, ZIF-1, PSD) have been effective, but these methods are limiting because they use a single input signal to control protein levels, preventing simultaneous differential control of multiple proteins with only one approach. Here we discuss the development of the LOCKR (Latching Orthogonal Cage–Key pRoteins) system for conditional protein depletion in *C. elegans*. There are two main components of LOCKR used for protein degradation: 1) the degronSwitch and 2) the inducible Key. The degronSwitch is fused to a protein of interest and, in the absence of Key, is in the "locked" state, caging the cODC degron. Upon interaction with Key, the degronSwitch is "unlocked." The exposed degron causes degradation of the Switch and any protein it is fused to via the proteasome. LOCKR Keys and degronSwitches can be designed as orthogonal pairs, allowing for the differential control of multiple proteins simultaneously. Here we describe our initial characterization of LOCKR in *C. elegans.* We fused degronSwitch to *dhc*-1, an essential cytoplasmic motor protein along with mScarlet and a 3xFLAG tag to aid in protein quantitation via microscopic and Western analysis. We also developed inert degronSwitch reporter via MosSCI for ubiquitous expression of the degronSwitch *in vivo* to examine the impact of the degronSwitch protein expression on worm health. To complement the degronSwitch, we designed tissue-specific Keys for MosSCI, which allows for spatial control of degronSwitch protein degradation. In addition to tissue specificity, the Key is also codon-optimized for *C. elegans* and fused to GFP and a 3xMyc tag for protein quantitation and visualization. Future work includes expanding the degronSwitch and Key pairs *in vivo* and incorporating temporal control of the degronSwitch fusion protein by toggling the Key on and off using heat-shock promoters and RNAi against the Key. This work establishes a novel and tissue-specific method for regulating protein function in *C. elega*ns.

599B **Regulation of transgenerational epigenetic H3K27me3 inheritance** Isa Ozdemir, Florian Steiner Department of Molecular and Cellular Biology, University of Geneva

Adaptation to external conditions is key to the survival of organisms. This may occur slowly but irreversibly through mutations, or dynamically epigenetic regulations. Epigenetics consists of regulatory systems controlling gene expression without any alterations in the DNA sequence, for example through the introduction and maintenance of histone post-translational modifications. However, our mechanistic understanding of how global patterns of histone modifications are maintained or reset across generations is incomplete.

Here, we investigate whether altered patterns of the heterochromatic histone modification H3K27me3 can be inherited across generations. To globally alter the distribution of H3K27me3 in germline nuclei, we take advantage of an H3.3K27M dominant negative mutant that inhibits PRC2 (the sole histone methyltransferase for H3K27me3) and restricts the deposition of this mark to the X chromosome and a few autosomal domains, leading to greatly reduced fertility [1]. By expressing H3.3K27M from an extrachromosomal array for one generation, we can study the effects on the genetically wildtype offspring.

We find that both the fertility defects and the aberrant H3K27me3 distribution are maintained in about 30% of the wildtype offspring, and when selecting for subfertile animals, these phenotypes are epigenetically inherited for at least 15 generations. Without selection, the phenotypes become undetectable among the wildtype offspring within five generations.

We identified two chromodomain proteins, CEC-6 and HERI-1, that play antagonizing roles in the epigenetic inheritance of the H3K27me3 patterns. Mutation of CEC-6 increases the stability of the epigenetic maintenance, resulting in about 60% of inheriting generations showing fertility and chromatin defects. Removal of HERI-1 destabilizes the epigenetic inheritance, and the majority of worms in the first wildtype generation show normal fertility and H3K27me3 patterns.

Our data supports a model where CEC-6 promotes the spreading of H3K27me3 incorporation and erases the epigenetic memory, while HERI-1 antagonizes the spreading of H3K27me3 incorporation and stabilizes the epigenetic memory, presumably through interaction with H3K23me3, a histone mark that transiently appears upon the H3.3K27M-mediated depletion of H3K27me3 genome-wide.

1. Delaney et al., H3.3K27M-induced chromatin changes drive ectopic replication through misregulation of the JNK pathway in C. elegans. Nat.Commun. (2019).

600B **A role for long non-coding RNAs in calcium signaling during embryogenesis and male mating** Vida Praitis[1], Julia Tlapa[2], Ian Johnson[2], Mary Frances Jarmusz[2], Yinan Hui[2], Yijun Xiong[2], Haonan Sun[2], Sam Kubica[2], Caleb Lee[21]Biology, Grinnell College, [2]Grinnell College

Calcium is vital for a plethora of cellular and physiological functions (Clapham 2007), so calcium concentrations are carefully regulated by pumps and transporters including the secretory pathway calcium ATPases (SPCA). In humans, mutations in a copy of the ATP2C1/SPCA1 pump result in Hailey-Hailey disease while disruptive mutations in both copies are likely lethal (Hu 2000; Sudbrak 2000). In *C. elegans*, the PMR-1/SPCA1 is a required in synuclein-related Ca++ cytotoxicity, (Buttner 2013), in maintaining calcium homeostasis during heat stroke (Kourtis 2012), in oxidative stress response (Cho, et al 2005), and for cell migration during embryogenesis (Praitis 2013). To identify genes that act with *pmr-1* in calcium homeostasis, we carried out a forward genetic screen to identify suppressors of the *pmr-1(ru5)* embryonic lethal phenotype. Our screen identified the *kez13* allele which acts maternally to suppress the embryonic lethal phenotypes in *pmr-1(ru5)* embryos. Whole genome mapping and sequencing identified *kez13* as a 5 kb deletion in the *sma-9* 5' UTR, which contains high-occupancy transcription factor binding sites (Liang, et al 2003; Chen, et al, 2014) and two long non-coding RNAs (Akay 2019). While our initial hypothesis was that suppression of *pmr-1(ru5)* lethality was due to impacts on *sma-9,* evidence indicates otherwise. The *kez13* allele does not produce a SMA phenotype, qPCR indicates *sma-9* mRNA expression levels are only modestly affected in *kez13* strains, and *sma-9(wk55)*; *pmr-1(ru5)* double mutants are not suppressed. Instead, a construct carrying the two long-non-coding RNAs in this genomic region, *linc-3290* and *linc-3291* (Akay 2019), is sufficient in *kez13* complementation analysis. Because the *kez13* deletion affects both genes, we built and tested RNAi constructs designed to disrupt just one. Our results are ambiguous, as RNAi disruption of either gene results in only modest suppression. Neither overexpression or loss-of-function alleles of *linc-3290* and *linc-3291* has significant embryonic lethal phenotypes in controls, but we do see suppression of lethality in *dbl-1(wk70)* and *sma-9(wk55)* strains, indicating that these lncRNA's may play complex roles during embryogenesis. In adults, the *kez13* strain has a male mating defect. While males are not sterile, and they can occasionally deliver sperm to produce cross progeny, they exhibit a high degree of ventral tail curling which negatively impacts their fertility.

601B **U6 snRNA m6A methylation is required for effective cis- and trans-splicing** Aykut Shen[1], Katarzyna Hencel[1], Matthew T Parker[2], Robyn Scott[3], Aduragbemi Adesina[1], Roberta Skukan[1], Tim Pearson[1], Eric A Miska[4], Wilfried Haerty[5], Yunsun Nam[3], Gordon G Simpson[2], Alper Akay[61]University of East Anglia, [2]University of Dundee, [3]The University of Texas Southwestern Medical Center, [4]University of Cambridge, [5]Earlham Institute, [6]School of Biological Sciences, University of East Anglia

RNA splicing is essential for the accurate expression of genes in many organisms. The most common form of RNA splicing is cis-splicing when introns are removed from pre-mRNAs. On the other hand, many organisms, including nematodes, flatworms, cnidarians, rotifers, euglenozoa and urochordates, use spliced leader (SL) trans-splicing to change the 5' ends of mRNAs. SL trans-splicing is essential for the accurate translation of mRNAs, and approximately 80% of mRNAs are SL trans-spliced in *C. elegans*. An important biological question is how the spliceosome recognises splice sites across various sequences. In cis-splicing, 5' splice sites are recognised first by U1 snRNA and then by U5 and U6 snRNAs, whereas the U2AF proteins recognise 3' splice sites which also require the branch site recognition by SF1 and U2 snRNA. SL trans-splicing requires the same snRNAs except for U1 and requires two separate RNA molecules to be brought together. Therefore, we don't know how the splicesome recognises 5' splice sites on SL RNAs. Using nanopore direct RNA sequencing, we show that U6 snRNA m6A methylation by the RNA methyltransferase METT-10 / METTL16 is required for efficient cis- and trans-splicing in *C. elegans.* U6 snRNA methylation is required during cis-splicing in *C. elegans* to effectively recognise 5' splice sites with adenosine in the +4 position. Furthermore, U6 snRNA methylation is essential for trans-splicing RNAs with weak 3' splice sites. Finally, we show that perturbation of trans-splicing can lead to the emergence of novel transcript isoforms.

602B **The Unknown unknowns: finding functions for poorly annotated genes in *C. elegans*** Amy Walker[1], Daniel Higgins[21]UMASS Medical School, [2]PMM, UMASS Medical School

In today's post-genomic era, we know that most metazoans genomes encode around 20K genes. It is striking that nearly half of these genes are sparsely annotated, and many others are described only by by domain conservation. This profoundly biases

gene enrichment studies, as the most well studied pathways have the most annotations per gene and limits studies into less well understood systems. For example, of 621 *C. elegans* transcription factors, the top 20 account for 40% of papers and have more than 2X the number of GO terms per gene.

We noted the scale of this issue when developing WormCat, a gene categorization tool for identifying and visualizing enrichment in genome-scale data, which depended on a custom annotation of the *C. elegans* genome. The WormCat annotation strategy differed from GO in two major ways. First, each gene received a single nested annotation with broad to more specific functions. These categories were based on physiological function, molecular or location-based category. Second, genes with little associated information were placed in a specialized category, UNASSIGNED. This allows poorly annotated genes (PAGS: UNASSIGNED and domain-based categories, such as TRANSMEMBRANE DOMAIN) to be tracked in transcriptomic experiments. We noted that nearly half the genes expressed in neurons or intestine from published studies were poorly annotated. One hypothesis is lineage specificity; however, we find that PAGs may have orthologs outside of *C. elegans*, which also lacked functional characterization. We find several indications that some of these genes may be amenable to functional characterization, such as phenotypes in large scale RNAi screens, which suggest functional testing has not been saturated. Using WormCat to interrogate a wide variety of published RNA seq data, we also find that UNASSIGNED genes may be enriched in specific tissues or stress conditions, suggesting response to specific regulatory cures. In order to classify these understudied genes, we are using machine learning tools to cluster PAGs with genes of known function for detailed phenotyping studies. Conserved but functionally uncharacterized genes may function outside the lab environment, contain redundant functions, or support robustness. Many -omics studies depend on pathway analysis or previous annotation to select genes for functional analysis. Developing tools to improve functional gene annotation is critical for evaluating -omics data and identification of the most biologically relevant candidates for future study.

603B **SAM synthase specific effects on histone methylation, gene expression and survival** Adwait Godbole[1], Alexander Munden[1], Dana Miller[2], Amy Walker[1,31]PMM, UMASS Medical School, [2]Biochemistry, University of Washington, [3]UMASS Medical School

Methylation is a widely occurring modification that requires the methyl donor S-adenosylmethionine (SAM) and acts in regulation of gene expression and other processes. SAM is synthesized from methionine, which is imported or generated through the 1-carbon cycle (1CC). Alterations in 1CC function have clear effects on lifespan and stress responses, but the wide distribution of this modification has made identification of specific mechanistic links difficult. We hypothesize that provisioning of SAM through specific synthases provides a level of regulatory specificity to methylation dependent pathways. In most animals, SAM may be synthesized by one of several SAM synthases. For example, mammals contain two SAM synthases, one of which is specific to adult liver (MAT1A). While MAT2A is ubiquitous, it may exist in multiple isoforms. The SAM synthase family in *C. elegans* is expanded, with 4 isoforms. *sams-1* is the most highly studied, with roles in lifespan extension, lipid storage and complex function in the stress response. Lowering SAM to similar levels by limiting *sams-4* is not sufficient to recapitulate these phenotypes, suggesting it is not the level of SAM, but how or where it is produced that provides the regulatory specificity. Tissue specific effects could explain some functional differences. However, using CRISPR tagged alleles, we find that SAMS-1 and SAMS-4 are largely co-expressed with the exception of the germline, which lacks SAMS-1.

We are leveraging the heat stress response to identify regulatory effects controlling differential effects. Reduction in these enzymes produces opposite phenotypes, distinct heat responsive gene expression programs, metabolic changes and H3K4me3 methylation patterns. We also find that SAMS-4 initiates a distinct histone methylation program in the absence of *sams-1* that impacts survival. Our preliminary data suggest SAMS-1 and SAMS-4 have some distinctions in their local proteomes, suggesting potential for distinct methylation targets or regulatory interactions. Taken together, our results suggest that the regulatory functions of SAM depend on its enzymatic source and that provisioning of SAM may be an important regulatory step linking 1CC function to phenotypes in aging and stress.

604B **Regulation of the period protein homolog LIN-42 by KIN-20** Collin Parrow[1], Benjamin Godbout[1], Michelle Coluzzi[1], Katherine McJunkin[2], Priscilla Van Wynsberghe[11]Biology, Colgate University, [2]NIDDK NIH

The *C. elegans* heterochronic pathway, which regulates developmental timing, is thought to be an ancestral form of the circadian clock in other organisms. An essential member of this clock is the Period protein whose homolog, LIN-42, in *C. elegans* is an important regulator of developmental timing. LIN-42 functions as a transcriptional repressor of multiple genes including the conserved lin-4 and let-7 microRNAs. Like other Period proteins, levels of LIN-42 oscillate throughout development. In other organisms this cycling is controlled in part by phosphorylation. KIN-20 is the *C. elegans* homolog of the *Drosophila* Period protein kinase Doubletime. Worms containing a large deletion in *kin-20* have a significantly smaller brood size, develop slower than wild type *C. elegans*, and display an uncoordinated phenotype. We have previously shown that KIN-20 impacts *lin-42* phenotypes. In addition, KIN-20 is important for post-transcriptional regulation of mature let-7 and lin-4 microRNA expression. However, the mechanisms by which KIN-20 regulates LIN-42 and microRNA biogenesis are unclear. Using epitope-tagged variants of LIN-42

and KIN-20, we have found that KIN-20 impacts LIN-42 expression. Current work aims to determine how KIN-20 causes these effects. Altogether, these findings further our understanding of the mechanisms by which these conserved circadian rhythmic genes interact to ultimately regulate rhythmic processes and developmental timing in *C. elegans*.

605B **BRC-1 and BRD-1 nucleosome ubiquitylation: conserved features and functional importance** Russell Vahrenkamp[1], Owen Falkenberg[1], Meenal Cascella[1], Mikaela D Stewart[21]TCU, [2]Biology Department, TCU

BRCA1 and BARD1 proteins heterodimerize in human cells to ubiquitylate nucleosomes aiding in DNA damage repair and gene repression. While this is hypothesized to contribute to tumor-suppression functions of the two proteins, the heterodimer also acts as a protein-protein interaction scaffold in many cellular processes leaving ambiguity about the molecular mechanisms that drive various BRCA1 functions. To address some of these remaining questions we are utilizing *C. elegans* to connect nucleosome ubiquitylation to functions in the context of a whole organism. We used *in vitro* biochemistry and *ex vivo* transcript quantification to establish that nucleosome ubiquitylation and gene repression are conserved in the *C. elegans* homologs, BRC-1 and BRD-1. We found that worm strains with mutated *brc-1* or *brd-1* overexpressed members of the cytochrome P450 (*cyp*) gene family that is most similar in sequence to *cyp* genes repressed by the human homologs. We also used purified proteins and reconstituted nucleosomes to show that while BRD-1 and BARD1 use different interfaces to interact with the nucleosome substrate, BRC-1 and BRCA1 have a conserved nucleosome binding interface that is vital for nucleosome ubiquitylation. In order to investigate the role that nucleosome ubiquitylation plays in the many functions of BRC-1 we generated a worm strain with a BRC-1 nucleosome-binding-deficiency allele in which all other protein interfaces of BRC-1 remain intact. We are currently using this strain to determine the importance of BRC-1 nucleosome ubiquitylation in meiotic X chromosome nondisjunction as measured by number of male self-progeny, generation of reactive oxygen species using an *in vivo* fluorescence assay, and repression of genes and microsatellite DNA as assayed by RT-PCR. We will share our findings regarding the conserved features of BRC-1 and BRD-1 nucleosome ubiquitylation and preliminary data regarding its contribution to the loss-of-BRC-1 phenotypes.

606B **A single-cell RNA-Seq strategy that uses combinatorial barcoding for whole transcriptome coverage of *C. elegans* neurons** Tyler Amos[1], Seth Taylor[1,2], Alec Barrett[3,4], Alexis Weinreb[3,4], Marc Hammarlund[3,4], David M Miller[11]Cell and Developmental Biology, Vanderbilt University, [2]Cell Biology and Physiology, Brigham Young University, [3]Department of Neuroscience, Yale University, [4]Department of Genetics, Yale University

The CeNGEN project seeks to produce gene expression maps for every neuron in the *C. elegans* nervous system in hermaphrodites and males and across post-embryonic development. Droplet-based sequencing methods are readily scalable and ideal for generating accurate profiles of single cells but typically do not provide whole transcriptome coverage (e.g., alternative splicing, noncoding RNAs). In contrast, bulk sequencing methods that rely on FACS-isolation of specific neuron types can achieve whole transcriptome coverage but are not amenable to high throughput data collection and are less accurate due to contaminating transcripts from non-target cells. We have now utilized an alternative approach that retains the precision of single cell sequencing while expanding transcriptome coverage. Mid-L1 larvae were dissociated with established methods and ~10,000 cells processed with a Parse combinatorial barcode labeling protocol. The Parse method uses both poly(dT) and random primers to target whole transcripts and noncoding RNAs. In addition to muscle, intestine, seam cell, glia, germ cells, etc., our data set includes ~40 clusters of identifiable neuron types. In comparison to a droplet-based profile of L1 cells, the Parse data set is more sensitive (3x UMI/cell) and affords robust 3'-5' transcript coverage. We are exploring a CAS9-based strategy for depleting ribosomal RNA sequences which comprise >60% of reads in the Parse data set.

607B **A single allele to determine the spatiotemporal expression pattern while also allowing for functional analysis of long non-coding RNAs** Sandeep N Wontakal[1,2], Katherine Maniates[3], Olivia N Wessenfels[4], Andrew Singson[3], Oliver Hobert[51]Pathology, Johns Hopkins University School of Medicine, [2]Pathology & Cell Biology, Columbia University Irving Medical Center, [3]Rutgers University, [4]Johns Hopkins University, [5]Columbia University

Long non-coding RNAs (lncRNAs) are a class of genes that are defined as non-coding transcripts that are ≥200 nucleotides. Genome analysis has identified >100,000s of such transcripts with some organisms having more lncRNAs than coding genes such as in humans. However, how many of these lncRNAs represent functional transcripts remains controversial. In *C. elegans*, >3,300 lncRNAs have been annotated that share many features of mammalian lncRNAs such as stage/cell-type restricted expression, suggesting *C. elegans* may provide a good model to study the roles of lncRNAs. Since many lncRNAs have stage/cell-type restricted expression, knowing the spatiotemporal expression of a lncRNA would help in choosing functional assays that can be used to assess loss-of-function (LoF) alleles.

To facilitate the study of lncRNAs in *C. elegans*, we developed the "NUll Transcriptional Reporter" (NuTR) cassette that can be used to replace the lncRNA locus via CRISPR – thereby generating a null allele – while simultaneously allowing the endogenous *cis*-regulatory elements of the lncRNA to drive the expression of a fluorescent protein in the cassette. This single allele

allows for determining the spatiotemporal expression of the lncRNA while also enabling the assessment of null phenotypes. A recently identified lncRNA, lep-5, was used to show our cassette was able to recapitulate the reported dynamic spatiotemporal expression pattern as well as the LoF phenotype. We next applied our NuTR cassette to 5 of the most male-enriched lncRNAs. We find unique expression patterns for the 5 lncRNAs that span all the cells of the male somatic gonad from spermatids through the vas deferens. We also find expression of 4/5 lncRNAs in the somatic gonad of hermaphrodites. Interestingly, some of the lncRNA are also expressed in a variety of other cell-types including, oocytes, glia, muscle, and hypodermal cells. Functional analysis finds 4/5 lncRNAs show a significant brood size defect, whereas none of the lncRNAs contribute significantly to the ability to generate cross progeny. In summary, we describe a method to generate a single allele that allows for determining the spatiotemporal expression pattern of lncRNAs and the functional effects of a null allele. We believe the NuTR cassette will aid in understanding the roles of lncRNAs in *C. elegans*. In addition, the cassette can easily be adapted to other ncRNAs, as well as coding genes.

608B **An anchored experimental design and meta-analysis approach to address batch effects in large-scale metabolomics** Lauren McIntyre[1], Amanda Shaver[2], Brianna Garcia[3], Goncalo Gouveia[4], Alison Morse[1], zihao liu[1], Cater Asef[5], Ricardo Borges[4], Franklin Leach[4], Erik Anderson[2], I Jonathan Amster[4], Facundo Fernandez[5], Arthur Edison[41]University of Florida, [2]Northwestern, [3]Woods Hole, [4]UGA, [5]Georgia Institute of Technology

Untargeted metabolomics studies are unbiased but identifying the same feature across studies is complicated by environmental variation, batch effects, and instrument variability. Ideally, several studies that assay the same set of metabolic features would be used to select recurring features to pursue for identification. Here, we developed an anchored experimental design. This generalizable approach enabled us to integrate three genetic studies consisting of 14 test strains of *Caenorhabditis elegans* prior to the compound identification process. An anchor strain, PD1074, was included in every sample collection, resulting in a large set of biological replicates of a genetically identical strain that anchored each study. This enables us to estimate treatment effects within each batch and apply straightforward meta-analytic approaches to combine treatment effects across batches without the need for estimation of batch effects and complex normalization strategies. We collected 104 test samples for three genetic studies across six batches to produce five analytical datasets from two complementary technologies commonly used in untargeted metabolomics. Here, we use the model system *C. elegans* to demonstrate that an augmented design combined with experimental blocks and other metabolomic QC approaches can be used to anchor studies and enable comparisons of stable spectral features across time without the need for compound identification. This approach is generalizable to systems where the same genotype can be assayed in multiple environments and provides biologically relevant features for downstream compound identification efforts. All methods are included in the newest release of the publicly available SECIMTools based on the open-source Galaxy platform

609B **The protective roles of heterochromatin in the response to stress and tissue integrity** Rosa Herrera Rodriguez[1], Valerie Arz[1], Stephen P Methot[2], Colin E Delaney[3], Jan Padeken[11]Institute of Molecular Biology, [2]Friedrich Miescher Institute for Biomedical Research, [3]Faculty of Natural Sciences, University of Basel

The epigenetic memory of a cell is shaped by the pathways that establish, erase, and maintain chromatin marks. Lysine 9 methylation on histone H3 (H3K9me) is a defining modification of heterochromatin. In multicellular eukaryotes, heterochromatin has two main functions. First, it silences repetitive sequences to ensure genome stability. Secondly, it maintains the silencing of genes during and post development, ensuring a stable differentiated state. The unprogrammed transcription of repetitive sequences leads to an accumulation of toxic R-loops and the dependence on BRCA1 and DNA repair proteins for survival. Thus, it is not surprising that a loss of appropriately targeted heterochromatin is associated with cancer and premature aging.

Here we show that the continuous deposition of H3K9me2 even after terminal differentiation is necessary to maintain repression, ensuring the silencing of non-lineage genes and tissue integrity. Surprisingly temperature stress triggers the global disruption of H3K9me deposition and the transient upregulation of heterochromatic sequences. This raises the intriguing possibility that specific tissues undergo a long-term adaptation to stress through changes in their epigenetic landscape

610B **Ribo-On and Ribo-Off: efficient manipulation of endogenous gene expression using a self-cleaving ribozyme** Jie Fang, Jie Wang, Yuzhi Wang, Xiaofan Liu, Baohui Chen, WEI ZOUZhejiang University

Investigating gene function relies on the efficient manipulation of endogenous gene expression. Currently, a limited number of tools are available to robustly manipulate endogenous gene expression between “on” and “off” states. In this study, we inserted a 63 bp coding sequence of T3H38 ribozyme into the 3’ UTR of endogenous genes using the CRISPR/Cas9 technology, which reduced the endogenous gene expression to a nearly undetectable level and generated loss-of-function phenotypes similar to that of the genetic null animals. To achieve conditional knock-out, a cassette of *loxP*-flanked transcriptional termination signal and ribozyme (*loxP*-stop-*loxP*-ribozyme) was inserted into the 3’ UTR of endogenous genes, which could eliminate gene expression spatially or temporally via the controllable expression of the Cre recombinase. Conditional endogenous gene turn-on can

be achieved by either injecting morpholino which blocks the ribozyme self-cleavage activity or using the Cre recombinase to remove the *loxP*-flanked ribozyme. Together, our results demonstrate that these ribozyme-based tools can efficiently manipulate endogenous gene expression both in space and time, and expand the toolkit for studying functions of the endogenous genes.

611B **Imaging transcriptional dynamics during development using a fluorescently-labelled Argonaute and its application to nested gene interactions** Cristina Vico Cantero, Fabien Soulavie, Vincent Bertrand, Antoine BarriereIBDM / CNRS / Aix-Marseille University

We have recently developed a method to image *in vivo* transcriptional activity by labelling active transcription sites using a fluorescently-labelled Argonaute protein, NRDE-3 (Toudji-Zouaz et al., Nucleic Acids Research 2021). Here we report the development of a semi-automated pipeline to extract fluctuations in transcription rates from movies acquired with this method. In addition, we are currently applying this approach to analyze the effect of a striking genomic configuration, the opposite nested gene arrangement, on the dynamics of transcription. Two protein coding genes are in an opposite nested configuration when a gene is located in one intron of another gene in an opposite direction. This arrangement is not infrequent, representing about 5 percent of protein-coding genes. Nested and host genes are sometimes expressed in the same cells. For example, the *ceh-10* gene, coding for a homeodomain transcription factor involved in neuronal specification, is entirely nested within an intron of *polq-1*, coding for a DNA polymerase, strongly expressed in the germline, but also concurrently in the same neurons expressing *ceh-10*. However, cotranscription of two genes on opposite strands at the same locus can cause interference, for example by collision of transcriptional complexes or DNA supercoiling. Using our method, we are testing whether coexpression of nested and host genes increases noise of expression with potential consequences on the robustness of gene activity.

612B **H4K20 Methylation Regulation in Cell Cycle and Dosage Compensation** Anati Alyaa Azhar[1], Jianhao Jiang[2], Györgyi Csankovszki[11]MCDB, University of Michigan, Ann Arbor, [2]University of Michigan, Ann Arbor

In *C. elegans*, hermaphrodites have two X chromosomes while males have a single X chromosome, leading to unequal gene expression. Dosage compensation is a process that solves this issue by equalizing this difference in gene expression between the two sexes. In C. elegans, hermaphrodites (XX) downregulate their X chromosomes by half to equalize it to males (X) via the Dosage Compensation Complex (DCC) in somatic cells. The DCC consists of a 5-subunit Condensin I^{DC} complex as well as additional accessory proteins that cooperate to downregulate the gene expression of the X chromosomes. One of the accessory subunits of the DCC is DPY-21, a H4K20-specific demethylase that enriches for H4K20me1 on dosage compensated X chromosomes which is the same chromatin mark enriched on mitotic chromosomes. We aim to understand the coordination between H4K20me1 regulation in dosage compensation and the cell cycle. In *C. elegans*, H4K20 methylation is affected by SET-1, a methyltransferase that places the first methyl mark on H4K20; SET-4, which converts H4K20me1 to di- and tri-methylation; and DPY-21, a non-condensin subunit of the DCC, which demethylates H4K20me2/3 to H4K20me1 on the X chromosomes. We show that in young embryos, where the cell cycle switches between M and S phases, DPY-21 and SET-4 play no role in the regulation of H4K20 methylation. However, we show that in late embryos which have cell cycles with gap phases, the two proteins begin to function. Even in these later cell divisions, much of the cell cycle regulation of H4K20 methylation remains intact in the absence of SET-4 and DPY-21, with a decrease in H4K20me1 levels in S phase on all chromosomes and an increase in H4K20me1 during mitosis on all chromosomes. SET-4 and DPY-21 have the greatest impact on H4K20me during G1. Our goal is to characterize the function and regulation of these two proteins by identifying their interactors and regulators.

613B **Optimizing genome-wide association approaches to determine the genetic basis of natural variation in starvation resistance** Jameson Blount[1], L. Ryan Baugh[21]Computational Biology and Bioinformatics, Department of Biology, Duke University, [2]Department of Biology, Center for Genomic and Computational Biology, Duke University

The physiological response to starvation is significant for its connection to metabolic disease, cancer, and aging. *C. elegans* L1 arrest is a system where the genetic and molecular interactions underlying the starvation response can be characterized. A better understanding of how starvation resistance is controlled, and its natural variation across wild strains, will be instrumental to gaining basic insights into physiology. Genome-wide association (GWA) software provides a starting place for experimental validation by using statistical modeling to generate a landscape of the genomic loci associated with a given trait. However, existing statistical models powering GWA software assume that individuals being studied are free of population structure that would confound the results. Population structure refers to systematic differences in allele frequencies between subpopulations due different ancestry, which can create false-positive signals of association. This structure is prevalent among *C. elegans* due to their primary existence as self-fertilizing hermaphrodites and low rate of ancestral recombination. These confounding factors make resolving the true causal variants difficult because jointly inherited variants create entire blocks of genomic regions that are statistically associated with the trait of interest, without revealing the true causal loci. Population structure can often be identified by these large blocks of linkage disequilibrium, since linked alleles become non-randomly associated due to low recombination that would normally disrupt ancestral haplotypes. Linear mixed models have found some success in adjusting for

these confounding factors by accounting for relatedness among samples, using a genomic relationship matrix (GRM) as a source of random effects in the model. Several strategies for constructing the GRM have varying results. We evaluate these strategies through simulation studies, designed to establish GWA performance benchmarks, and genomic inflation, which estimates the amount of inflation in GWA results by comparing the distribution of observed test statistics across all genetic variants to that expected under the null hypothesis. Additionally, we capture more information to inform the GWA model by harnessing the experimental strain-specific variance from trait measurement. By implementing these changes, we hope to improve how genetic variants are mapped to changes in phenotypic expression of polygenic traits in *C. elegans*.

614B **Investigating the role of chromatin structure in starvation resistance via novel histone variant *hil-1/*H1-0 using *C. elegans*** Kinsey Fisher[1], Rojin Chitrakar[2], Ryan Baugh[31]Duke University, [2]Biology, Duke University, [3]Biology, Duke University

Insulin/IGF signaling (IIS) is a critical pathway in biomedical research for its relevance in cancer, ageing, and metabolic disease. *Caenorhabditis elegans* have the fascinating ability to arrest development to survive starvation through activation of the transcriptional effector of IIS, *daf-16/*FoxO, whose activation inhibits pathways that promote development and activates stress-response genes. Although *daf-16*-dependent changes in gene expression are well documented, the mechanisms behind this large-scale shift in gene expression remains unclear, and the role that chromatin structure plays to promote developmental arrest and starvation resistance has yet to be explored. We have identified linker histone variant *hil-1/*H1-0 as a positive target of *daf-16/*FoxO during starvation. *hil-1/*H1-0 is one of 7 somatic H1 histones in *C. elegans,* but the only one with expression specific to starvation. The general structure of H1 histones is highly conserved across metazoa, but the sequence and expression of variants is diverse. Although the mammalian ortholog H1-0 has been implicated in differentiation, repression of gene expression and tumor cell proliferation, *hil-1*/H1-0's role in starvation remains unknown. Preliminary assays revealed that *hil-1/*H1-0 promotes starvation resistance and represses genes involved in growth and development. I hypothesize that HIL-1 has evolved a specialized function from other H1 variants by condensing chromatin in a starvation-specific manner, repressing developmental gene expression to promote arrest. I am using a multi-omics approach to understand how HIL-1 alters chromatin structure and gene expression during starvation. I will also conduct phylogenetic and functional comparisons to other H1 variants, such as *hil-2*, which is expressed during development and repressed during starvation, to better understand the evolutionary significance of this gene. Identifying the role of *hil-1/*H1-0 in the genetic landscape in response to starvation will advance our understanding of how organisms evolved to cope with nutrient stress via IIS and have implications for aging, cancer and metabolic disease.

615B **L1 starvation gene-expression atlas at single-cell resolution** Jingxian Chen[1], Rojin Chitrakar[1], Ryan Baugh[1,21]Biology, Duke University, [2]Center for Genomic and Computational Biology, Duke University

The nematode *Caenorhabditis elegans* lives a "famine or feast" life in the wild and therefore has a variety of signaling and gene-regulatory responses to survive starvation. When *C. elegans* embryos hatch in the complete absence of food, they enter a developmentally arrested phase called L1 arrest. During L1 arrest, worms exhibit drastic changes in their transcriptome compared to that of fed animals, and key regulators of L1 starvation response have been characterized by RNA-seq at the whole-organism level. However, *C. elegans* is a multicellular animal, with each cell type displaying distinct features and executing unique functions. Transcriptomic differences that underlie those variations are averaged out by bulk RNA-seq, which calls for studies that characterize the L1 starvation response with a finer anatomical granularity. To address this problem, we applied single-cell RNA-seq to synchronized populations of L1s that are either 12 h into starvation or 6 h post hatching with food at 20°C from 7 biological replicates. The rationale for picking these time points is that the transcriptional response to starvation reaches a steady state within 12 h and most cell lineages start dividing in fed larvae after 6 h, hopefully avoiding confounding effects of nutrient availability and development. We have optimized 3' UTR annotation and compared it to existing annotations, optimized low-quality cell filtering, and compared different approaches to cell cluster annotation. We are currently investigating cell type-specific regulation of metabolism. Our study addresses fundamental questions regarding cell type-specific responses to nutrient availability and establishes a gene-expression atlas of fed and starved L1 larvae.

616B **Sensory neuron transcriptomes reveal complex neuron-specific function and regulation of mec-2/Stomatin splicing** Canyon K Calovich-Benne[1], Xiaoyu Liang[21]Biological Sciences, Southern Methodist University, [2]Southern Methodist University

The function and identity of a cell is shaped by transcription factors controlling transcriptional networks, and further shaped by RNA binding proteins controlling post-transcriptional networks. To overcome limitations inherent to analysis of sparse single-cell post-transcriptional data, we leverage the invariant Caenorhabditis elegans cell lineage, isolating thousands of identical neuron types from thousands of isogenic individuals. The resulting deep transcriptomes facilitate splicing network analysis due to increased sequencing depth and uniformity. We focus on mechanosensory touch-neuron splicing regulated by MEC-8/RBPMS. We identify a small MEC-8-regulated network, where MEC-8 establishes touch-neuron isoforms differing from default isoforms found in other cells. MEC-8 establishes the canonical long mec-2/Stomatin isoform in touch neurons, but surprisingly the non-canonical short isoform predominates in other neurons, including olfactory neurons, and mec-2 is required for olfaction. Forced

endogenous isoform-specific expression reveals that the short isoform functions in olfaction but not mechanosensation. The long isoform is functional in both processes. Remarkably, restoring the long isoform completely rescues mec-8 mutant mechanosensation, indicating a single MEC-8 touch-neuron target is phenotypically relevant. Within the long isoform we identify a cassette exon further diversifying mec-2 into long/extra-long isoforms. Neither is sufficient for mechanosensation. Both are simultaneously required, likely functioning as heteromers to mediate mechanosensation.

617B **Scaffold protein interactions govern embryonic mRNA decapping condensates** Elva Vidya[1], Yasaman Jami-Alahmadi[2], Adarsh Mayank[2], Tianhao Cheng[1], James Wohlschlegel[2], Thomas Duchaine[11]Department of Biochemistry, Goodman Cancer Research Institute, McGill University, [2]Department of Biological Chemistry, David Geffen School of Medicine, University of California Los Angeles

mRNA decapping is catalyzed by DCAP-2 (Dcp2) and promoted by co-factors that converge in -and often scaffold- cytoplasmic mRNA-Protein (mRNP) condensates called P bodies. Decapping co-factors are differentially enriched in somatic and germline blastomeres in the embryos, and yet the mechanisms governing their expression remain elusive. Our proteomic surveys indicate that loss of specific decapping co-factors EDC-3 and EDC-4 alter DCAP-2 association with the germline-enriched CGH-1/CAR-1/IFET-1 decapping-promoting complex. Our current results support a model wherein DCAP-2 interaction with EDC-3 and EDC-4 promote the somatic clearance of CGH-1/CAR-1/IFET-1 complex involving successive events of mRNA and ubiquitin-mediated proteasomal degradation.

618B **The expanded role of the conserved *snpc-1* and *snpc-3* gene families in *C. elegans* small RNA transcription** Lars K Benner[1], Rebecca Tay[2], Margaret R Starostik[2], Ayaka Inoki[2], Mindy Clark[2], John Kim[21]Biology, Johns Hopkins University, [2]Johns Hopkins

PIWI-interacting RNAs (piRNAs) are a class of small RNAs that have a conserved function in protecting the germline genome from the deleterious effects of mobile DNA elements. In repressing these elements, piRNAs preserve the integrity of the genome and ensure its faithful transmission to the next generation. While the transposon silencing function of piRNAs is well understood, the transcriptional regulation and sexual dimorphic expression of piRNAs remain largely unknown. The conserved snRNA activating protein complex (SNAPc) is a well-established transcription factor complex that drives small nuclear RNA (snRNA) transcription. In flies and mice, the SNAPc holocomplex consists of SNPC-1, SNPC-3, and SNPC-4 subunits, which are each encoded by a single gene. In contrast, the *C. elegans snpc-1* and *snpc-3* genes have been amplified through gene duplications to comprise several paralogs, each with distinct roles. We previously showed that the SNPC-1 family protein SNPC-1.3 is a male piRNA transcription factor expressed in the male germline. Here, we provide biochemical and genetic evidence to show that the SNPC-1 paralog SNPC-1.2 constitutes a novel female piRNA transcription factor. Additionally, genetic knockout and RNAi-based knockdown assays reveal that the *snpc-3* family genes *snpc-3.1* and *snpc-3.2* comprise functionally redundant core piRNA transcription factors required for the transcription of both male and female piRNAs, while *snpc-3.4* is uniquely involved in snRNA transcription. Collectively, the *snpc-1* and *snpc-3* gene families encode specificity factors for three distinct protein complexes that discriminate between the transcription of sex-specific piRNAs and snRNAs in the *C. elegans* genome. Our work provides insights into the sexually dimorphic, piRNA-mediated regulation of germline genes to maintain proper germline development and suggests that piRNA biogenesis emerged from the diversification of the ancient snRNA transcriptional machinery.

619B **Maternal effects of post-embryonic neuroblast migration in C-mannosyltransferase mutants** Hoikiu Poon, Chaogu Zheng The University of Hong Kong

Maternal effect is a phenomenon in which the phenotype of an offspring is determined by the mother's genotype instead of its own genotype or the environment (Wolf & Wade, 2009). Maternal effect usually affects processes involved in embryogenesis and is rarely found to regulate post-embryonic development. We found one exception in *Caenorhabditis elegans* when investigating a mutation that affects the post-embryonic differentiation of the Q neuroblasts and found that the defects in Q migration showed maternal rescue, i.e. homozygous mutants derived from a heterozygous mother did not display the phenotypes. This mutation was then mapped to *dpy-19*, whose loss led to the anterior displacement of the QL descendants, including PVM, SDQL, and PQR neurons. *dpy-19* codes for a C-mannosyltransferase (Buettner et al., 2013). We further quantified the phenotypes of our allele with other reference alleles (*e1259*, *e1314*) of *dpy-19* and found that they all showed similar maternal effects. We also screened many genes known to regulate Q neuroblast migration (Middelkoop et al., 2012; Buettner et al., 2013; Sundararajan et al, 2012, 2014), but only *dpy-19* shows maternal effects, suggesting a unique genetic property for *dpy-19*. Importantly, the *dpy-19(-)* phenotype was also zygotically rescued, meaning that heterozygous offspring from a homozygous mutant mother did not show the defects.

We explored the potential pathways for which maternal influence can affect offspring phenotype. Using endogenous GFP knock-in, we found that the DPY-19 proteins were not deposited to the oocytes. However, single molecule fluorescence in situ hybrid-

ization (smFISH) showed maternal deposition of *dpy-19* mRNAs, which were detected in early embryos. We are in the process of understanding the mechanism behind the long-term stabilisation of *dpy-19* mRNA throughout embryogenesis and early larval stages by investigating the possible involvement of RNA modification in the process, as well as the role of the 3'UTR of *dpy-19*.

620B **Dynamic evolution of telomeric-repeat motifs in the phylum Nematoda** Jiseon Lim[1,2], Wonjoo Kim[1,2], Jun Kim[3,4], Junho Lee[1,2,41]Department of Biological Sciences, Seoul National University, [2]Institute of Molecular Biology and Genetics, Seoul National University, [3]Department of Convergent Bioscience and Informatics, Chungnam National University, [4]Research Institute of Basic Sciences, Seoul National University

Telomeres are made up of tandem arrays of telomeric-repeat motifs (TRMs) and telomere-binding proteins (TBPs), which address the difficulties of end-protection and end-replication. TRMs are usually highly conserved due to the sequence specificity of TBPs, although significant TRM alterations have been observed in several taxa but not in Nematoda. We used public whole-genome sequencing data to analyze putative TRMs of 100 nematode species to investigate TRM evolution in Nematoda. We discovered that five distinct branches included specific novel TRMs, suggesting frequent TRM evolution in Nematoda. We concentrated on one of the five branches, the Panagrolaimidae family, to validate TRM evolution by collecting nematode isolates and obtaining whole-genome sequencing data. We also *de novo* assembled four high-quality draft genomes of Panagrolaimidae isolates and these genomes showed densely clustered arrays of the novel TRM. With reference to the telomere evolution in *Caenorhabditis elegans*, we comprehensively analyzed subtelomeric regions in the genomes to determine how the unique TRM evolved. We found that the novel TRM was used to preserve telomere integrity by alternative lengthening of telomeres even in isolates that employ the canonical TRM. We propose a hypothetical scenario in which some pre-existing TBPs may be capable of binding both canonical and novel TRMs, resulting in pre-adaptation of the novel TRM and aiding its evolution in the family Panagrolaimidae.

621B **Systematic identification and quantification of RNA modifications in *Caenorhabditis elegans* by ultraperformance liquid chromatography coupled to mass spectrometry** Gefei HUANG[1], Yiming Ma[2], Dongying Xie[2], Zongwei Cai[1], Zhongying Zhao[21]Department of Chemistry, Hong Kong Baptist University, [2]Department of Biology, Hong Kong Baptist University

Cellular RNAs carry more than 100 types of chemical modification. These modifications can be found across all types of cellular RNA species including mRNAs, rRNAs, tRNAs and small RNAs. Previous genetic and biochemical studies revealed the importance of RNA modifications in the normal physiology and stress response of *C. elegans*. Although Oxford Nanopore direct RNA sequencing of *C. elegans* transcriptome suggested an unexpected complexity of putative modifications in the mRNA transcripts, the identity, occurrence, and abundance of RNA modifications in *C. elegans* remain poorly understood. In this study, using ultra-performance LC (UPLC) coupled to complementary mass spectrometry (MS) techniques, i.e., high-resolution Orbitrap-MS and Triple stage quadrupole MS, we were able to identify 36 different types of RNA modification in the *C. elegans* total RNAs, 20 of which were quantified with the aid of standard modified ribonucleosides. Our data suggested that the abundance of modified ribonucleosides in small RNAs (18.46%) was significantly higher than that of noncoding RNAs (2.30%) and mRNAs (0.81%). We identified and quantified 17 different modifications in worm mRNAs, such as monomethylated guanosines (i.e., m^7G at level of 1.5 per 10,000 nt, m^1G at level of 0.6 per 10,000 nt, and m^2G at level of 0.2 per 10,000 nt), four types of ribose methylation, (i.e., Am, Cm, Gm, and Um with occurrence ranging from 3.8 – 12.6 per 10,000 nt), two types of modification on adenosine base, (i.e., m^1A and m^6A each with 1.4 per 10,000 nt) and I at 1.9 per 10,000 nt. The most abundant modification in mRNA pool is pseudouridine (34.6 per 10,000 nt). Given the significance of RNA modifications in gene expression regulations and epigenetic control, our study provides an entry point for transcriptome-wide mapping and functional analysis of RNA modifications in *C. elegans* development and physiology.

622B **Investigating the role of the chromatin remodeller ATRX/XNP-1 in *C. elegans*** Janie C Olver[1], Karim Hussain[1], Peter Sarkies[2], Helder C Ferreira[11]Biomedical Sciences Research Complex, University of Andrews, [2]Department of Biochemistry, University of Oxford

ATRX is a member of the SWI/SNF family of ATP-dependent chromatin remodelling enzymes. Its mutation causes the neurodevelopmental condition ATRX syndrome; is strongly linked to alternative lengthening of telomeres (ALT) positive cancers and leads to the de-repression of retrotransposons. However, the precise molecular function of ATRX or why its mutation causes such disparate phenotypes is currently unknown. We find that mutation of its *C. elegans* homologue, XNP-1, also results in developmental defects; upregulation of ALT markers, namely telomeric C-circles at 25°C and de-repression of retrotransposons. We tested the hypothesis that it is the de-repression of retrotransposons that subsequently drives the developmental and telomeric phenotypes. By focussing on the Cer10 LTR retrotransposon, we find XNP-1 silences Cer10 differently to how retrotransposons are repressed in mammalian cells. That is that XNP-1 represses retrotransposons independently of the histone mark H3K9me3, the histone variant H3.3 and nuclear RNAi. However, de-repression of retrotransposons is probably not sufficient to explain the developmental and telomeric phenotypes seen in *xnp-1* mutants, as the de-repression of retrotransposons does not correlate with a marker of general health, brood size. We wondered then whether XNP-1 is involved in regulating the expression of other

genes and whether aberrant transcription of these genes causes the observed developmental and telomeric phenotypes. By analysing transcriptional changes in *xnp-1* mutant embryos and L1s, we see aberrant upregulation of germline-specific genes. This raises the exciting possibility that XNP-1's function is to safeguard cellular identity and that the developmental and telomeric phenotypes observed in *xnp-1* mutants are due to increased germline character within the soma.

623B **Neuronal sensation drives transgenerational small RNA inheritance** Guy Teichman[1], Mor Sela[2], Itai Rieger[1], Hila Doron[2], Vladyslava Pechuk[3], Yael Mor[2], Sarit Anava[1], Meital Oren[3], Oded Rechavi[11]Neurobiology, Tel Aviv University, [2]Tel Aviv University, [3]Weizmann Institute

In Caenorhabditis elegans nematodes, small RNAs can be affected by environmental conditions and transmitted from parents to progeny for multiple generations, leading to transgenerational regulation of gene expression. Recent work has shown that small RNAs synthesized in neurons can regulate germline small RNA pool, germline gene expression, and behavior, in a transgenerational manner. This raises an intriguing question - can neuronal sensation of environmental changes trigger transgenerational epigenetic inheritance?

Here, we show that manipulating temperature sensation in the AFD thermosensory neurons via classical and chemo-genetic approaches drives transgenerational changes in small RNA-mediated silencing in the germline. This occurs even in the absence of temperature change. Moreover, we show that mutations in temperature sensation and signalling pathways modifies the dynamics of small RNA inheritance in response to temperature change. Finally, we suggest a model that describes how temperature sensation in the AFD neurons mediates heritable small RNA responses.

Together, our results indicate that neuronal sensation on its own can induce transgenerational epigenetic changes. Due to the ability of the nervous system to integrate environmental information over time, this could protect from environment-irrelevant heritable gene regulation.

624B **Bioinformatic identification of *Pristionchus pacificus* epigenetic genes reveals the evolutionary loss of the histone methyltransferase PRC2** Audrey Brown[1], Cameron Weadick[2], Yani Meiborg[3], Madelyn Purnell[1], Michael S Werner[11]Biology, University of Utah, [2]University of Exeter, [3]EMBL

The nematode *Pristionchus pacificus* has arisen as a model system for phenotypic plasticity since it displays an environmentally sensitive mouth-form phenotype. Recent work suggests that the environment may interact with the epigenetic landscape to regulate the expression of key mouth-form switch genes. However, the cast of epigenetic proteins in *P. pacificus* is not yet well characterized. Here, we use orthology-based analyses to produce comprehensive and comparative *P. pacificus* and *C. elegans* epigenetic gene datasets. Broadly, we observed a similar number of genes in each epigenetic category. However, the typically conserved histone methyltransferase PRC2 (polycomb repressive complex 2) is absent in *P. pacificus*. We also performed LC-MS/MS on semi-purified histones to define the range of histone modifications observed in *P. pacificus.* Though we cannot find genetic evidence of PRC2, we observe its cognate mark (H3K27me3). Finally, we define the selective pressures behind the loss of the catalytic PRC2 protein EZH2 and demonstrate that it was lost once in the *Pristionchus* lineage due to a local deletion event. Thus, the absence of PRC2 in *P. pacificus* is an opportunity to investigate the mechanisms and evolutionary pressures behind the loss of typically conserved genes.

625B **Gene co-expression network analysis identifies regulators of activity-regulated gene expression patterns in the AFD thermosensory neurons** Samuel G Bates, Nathan Harris, Piali SenguptaBrandeis University

Neuronal activity drives temporal waves of gene expression via regulatory mechanisms that remain to be fully elucidated. The AFD thermosensory neuron pair in *C. elegans* is an ideal model for studying these pathways. AFD responds to temperature (*T*) changes at a *T* experience-dependent threshold. This threshold is plastic and is altered based on the animal's cultivation *T*. Response plasticity is achieved in part by gene expression changes in AFD. We recently showed that gene expression induced by *T* experience follows distinct temporal patterns in AFD (see abstract by Nathan Harris). To characterize these patterns and identify the underlying regulatory mechanisms, we performed AFD TRAP-Seq after shifting animals from cool to warm *T* for different time periods. We then used weighted gene co-expression network analysis to categorize *T*-regulated genes into co-expression modules, each of which exhibits distinct temporal expression dynamics. Reasoning that promoters of co-expressed genes share binding motifs for transcription factors (TFs) responsible for their temporal co-regulation, we performed motif enrichment analysis on genes in individual modules. This analysis showed enrichment of CREB/CRH-1 motifs in the promoters of genes which are rapidly upregulated upon a *T* upshift. Using endogenously tagged reporters we have validated that the *T*-regulated expression changes of these genes are lost in *crh-1* mutants, and identified CREB binding sites in their promoters. In addition to CREB, we found identified motifs of other known regulators of neuronal gene expression enriched in the promoters of rapidly upregulated genes, suggesting that multiple TFs including CREB operate in AFD to direct rapid *T*-dependent upregulation of gene expression. We also identified enrichment of motifs of rapidly upregulated TFs as well as additional TFs within

the promoters of genes in modules which exhibit slower and/or sustained upregulation. We are currently working to validate a link between fast and slow co-expression modules, and to identify the roles of these additional TFs. Further analysis and *in vivo* validation of this gene regulatory network will allow us to define the regulatory events that translate a transient stimulus into both short- and long-term transcriptional and functional changes in a single sensory neuron type.

626B **Investigating the mechanism of heterochromatin sequestration by the euchromatin reader MRG-1: a role for mitochondrial stress?** Carole Zaratiegui, Daphne S. Cabianca Helmholtz Munich

Eukaryotic genomes are packaged within the nucleus thanks to a regulation of chromatin organization at multiple levels. Based on its transcriptional activity, chromatin is divided into euchromatin which is transcriptionally permissive, and transcriptionally repressed heterochromatin. Notably, eu- and heterochromatin are spatially segregated, with euchromatin being centrally located and heterochromatin sequestered around the nucleolus and at nuclear periphery, allowing for a functional compartmentalization of the genome. Perinuclear sequestration of heterochromatin occurs at the nuclear lamina via an H3K9 methylation-centered pathway, which is predominant in embryos. Recently, a new, H3K9 methylation independent, pathway of heterochromatin peripheral anchoring has been identified in *C. elegans* intestinal cells. This requires the H3K36 methylation reader MRG-1, whose loss leads to a gain of function of the histone acetyltransferase CBP-1, which is sufficient to promote heterochromatin internalization (Cabianca et al., 2019). However, the mechanisms through which MRG-1 loss leads to a CBP-1 gain of function remains unknown. The transcriptomic profile of *mrg-1* null animals reveals the activation of a stress response, raising the fascinating possibility that a stress cascade is responsible for the CBP-1 gain of function in *mrg-1* mutants and ultimately for heterochromatin detachment. Here, using a well-established heterochromatin reporter, we show that i) impairment of the mitochondria electron transport chain complex III and ii) knocking down inhibitors of the MAPK pathway causes heterochromatin detachment from nuclear periphery in intestinal cells, mimicking *mrg-1* mutant phenotype. Moreover, a mutation in the largely uncharacterized, mitochondria surveillance factor F40F12.7 rescues heterochromatin detachment by MRG-1 loss. With this work, we are contributing to understanding the role of stress signaling pathways in 3D chromatin architecture within tissues of an intact organism.

627B **Developing a quantitative gene network for the epidermal stem cells of *C. elegans*** Alicja Brozek[1,2], Arianna Ceccarelli[3], Mark Hintze[1], Andreas Joergensen[2], Vahid Shahrezaei[2], Michalis Barkoulas[11]Life Sciences, Imperial College London, [2]Mathematics, Imperial College London, [3]Mathematics, University of Oxford

Understanding stem cell regulation is essential for our understanding of development and regenerative medicine. *C. elegans* contains a set of epidermal stem-like cells known as seam cells. While the behaviour of these cells is well-characterised, the underlying gene network remains to be elucidated. We focus here on three genes that are thought to be the core transcription factors (TFs) of the seam cell gene network. These are the GATA factors *elt-1* and *egl-18* and the *engrailed* homolog *ceh-16.* To develop a quantitative understanding of the relationships between these three TFs and the architecture of the gene network, we used single molecule Fluorescent *in Situ* Hybridisation (smFISH) data and quantified gene expression in individual seam cells in the late L1 stage which we considered a steady state time point. We then evaluated all the possible links between the genes using the Modular Response Analysis (MRA) algorithm, which allowed us to predict the strengths of all the possible interactions of the network. To reduce the number of connections to just the ones that are essential for the correct function of the network, we utilised the Gibbs algorithm, which evaluates the significance of each connection within the network . Finally, we validated the derived network model by comparing our theoretical predictions to experimental quantification of the expression levels of all three TFs in the double mutant *ceh-16(bp323);egl-18(ga97)* background. We further tested our model in the context of other time points to identify changes in the seam cell gene network behaviour during development. This combination of experimental and theoretical approaches allowed us to predict a core seam cell gene network architecture and evaluate its behaviour over the course of development.

628B **Identifying the genetic network of functional components of constitutive heterochromatin and deciphering its relationship with other nuclear processes.** Anna F Townley, Roopali Pradhan, Julie Ahringer The Gurdon Institute, University of Cambridge

Constitutive heterochromatin makes up 20 to 50% of animal genomes and is an important modulator of development. It is enriched for repressive histone modifications H3K9me2/3 and associated with the silencing of gene expression and repetitive elements. The mechanisms through which heterochromatin is established and maintained are not well understood.

We previously identified a network of five heterochromatin factors that co-localise with H3K9me2 at repetitive elements and other genomic regions (MET-2, SET-25, LET-418, LIN-13 and HPL-2*;* McMurchy et al, 2017*).* These factors regulate transposable element repression, gene expression, DNA repair, and fertility and growth*,* in close collaboration with small RNA pathways. To identify and enable functional dissection of further components of the heterochromatin network, we carried out genetic inter-

action RNAi screens, leveraging the observation that genetic interactions occur between all double mutant combinations.

To this end, we screened a panel of 2309 RNAi clones targeting genes encoding nuclear proteins for phenotypic interactions with eight heterochromatin mutants: H3K9 methyltransferases (*met-2, set-25),* HP1 orthologues (*hpl-1, hpl-2*) and HP1-interacting proteins (*lin-61, lin-13, tdp-1*). We identified 289 enhancers and 89 suppressors of the phenotypes of one or more mutants. Most hits interacted with more than one strain, emphasising the involvement in shared processes.

Genetic enhancers include transcription and chromatin regulatory factors, and components of ubiquitylation, SUMOylation, ribosome and nucleolar biogenesis, and RNA regulatory pathways. Many genetic suppressors encode proteins associated with active transcription or chromatin modification. In follow up work on the enhancers, we identified components of ubiquitin ligase pathways required for heterochromatin reporter silencing. We also found that interactions with nucleolar processes involve activation of the integrated stress response pathway (see abstract by Pradhan et al). We are currently investigating the effects of enhancers and suppressors on gene expression to understand the nature of the interactions and to query whether they act through common mechanisms.

Our heterochromatin genetic network has identified new functional players in metazoan heterochromatin and gives insights into the interactions with a wide range of nuclear processes and chromatin-modifying pathways.

629B **Identifying and Characterising the Protein Composition at H3K9me3 in *C. elegans*** William Smith[1], Valeryia Aksianiuk[1], Devanarayanan Siva Sankar[2], Jörn Dengjel[2], Rodrigo Villasenor[3], Tuncay Baubec[4], Peter Meister[11]Institute of Cell Biology, University of Bern, [2]Department of Biology, University of Fribourg, [3]Biomedical Center LMU Munich, [4]Department of Biology, University of Utrecht

The DNA within the eukaryotic nucleus is packaged and folded through its association with various proteins, collectively known as chromatin. Chromatin can be categorized into two types: euchromatin, which is loosely folded and accessible to transcriptional machinery, and heterochromatin, which is densely folded and less accessible. The nucleosome, consisting of 147bp wrapped around a histone octamer, is the basic repeating unit of chromatin and is a key regulator of chromatin state. Post-transcriptional modifications (PTMs) of histone tails determine chromatin state and serve as recruitment sites for cellular machinery that remodel chromatin. To understand how chromatin states are achieved, we must identify the proteins responsible for depositing, erasing, and maintaining histone PTMs, as well as those that act downstream to achieve chromatin functional remodeling. To address this question, we used ChromID, a proximity biotinylation tool, in *C. elegans* to identify the protein composition at trimethylated H3K9 (H3K9me3), a modification associated with constitutive heterochromatin. Constitutive heterochromatin represses repetitive elements, some tissue-specific genes, and anchors heterochromatin at the nuclear periphery. Dysregulation of H3K9me3 is associated with several diseases, including neurodegeneration, cancer and aging. Using ChromID, we identified several known binders of H3K9me3, such as CEC-4 and HPL-2, as well as novel factors like VRK-1, CEC-5 and HMG-11. To investigate these hits further, we performed RNAi in larvae against each hit with an established heterochromatin reporter to screen for both derepression and delocalization from the nuclear periphery. Our preliminary results suggest that CEC-5 is a strong candidate for both delocalization and derepression. Interestingly, RNAi against VRK-1 and an unstudied protein, Y73B3A.1, show increased repression upon knockdown. If reproducible, we aim to combine fluorescent microscopy, genome-wide approaches, and phenotypic assays to further characterize these factors in regard to heterochromatin. Overall, we plan to apply ChromID to different histone modifications, mutant backgrounds, and tissue-specific contexts. By identifying various factors associated with H3K9me3, we hope to drive further research in the field of heterochromatin in C. elegans, which can be applied to mammalian systems.

630B **Robust regulation of QR neuroblast descendant migration through an activator-based timing mechanism** Lucia Garcia del Valle[1], Erik S Schild[2], Andrew M Mugler[3], Rik Korswagen[11]Hubrecht institute, [2]Hubrecht Institute, [3]Department of Physics and Astronomy, University of Pittsburgh

Many developmental processes rely on precise temporal control of gene expression. To achieve high temporal precision, the inherent noise of biological processes such as transcription needs to be overcome. When a biological timer consists of multiple cells, timing precision can be increased through averaging and synchronization. However, high temporal precision can also be achieved in individual cells, but the underlying cell intrinsic regulatory mechanisms are still poorly understood.

Using mathematical modelling, we found that a non-linear increase in the expression of a timekeeper gene through the accumulation of a transcriptional activator or decay of a transcriptional repressor can achieve higher temporal precision than an unregulated system. To study this in a tractable *in vivo* system, we turned to the QR neuroblast lineage of *C. elegans.* During its progression, QR.p and its daughter QR.pa migrate anteriorly until QR.pa reaches its final position and stops. We have previously shown that this is a temporally regulated process, in which the time-dependent expression of the Wnt receptor *mig-1*/Frizzled triggers a switch in Wnt signalling response that stops migration.

Consistent with the non-linear increase predicted by our model, *mig-1* expression is sharply upregulated in QR.pa. Furthermore, using CRISPR/Cas9 mediated genome editing to delete conserved cis-regulatory elements in the *mig-1* locus, we found that this upregulation is dependent on transcriptional activation. Together, these results provide experimental support for our model that temporal precision is achieved through an accumulating transcriptional activator that triggers expression of the regulated gene once a specific threshold is crossed.

Here, we identify the transcriptional activator that controls the time-dependent expression of *mig-1*. Using ChIP-seq data, we found that the Hox transcription factor LIN-39 binds a region in the *mig-1* promoter that is essential for expression in QR.pa. Consistently, *mig-1* expression is lost in *lin-39* mutants. Importantly, smFISH analysis showed that *lin-39* expression precedes the upregulation of *mig-1*, which is consistent with its role as an accumulating activator. Characterization and manipulation of LIN-39 protein levels will provide further insight into the temporal regulation of *mig-1* expression. This will help us understand how single-cell timers are capable of cell-autonomously controlling developmental processes with high temporal precision.

631B **Cer1 Virus-like Particles mediate horizontal and vertical transmission of epigenetically encoded environmental information** Renee Seto[1], Jasmine Ashraf[1], Rachel Kaletsky[1], Coleen T Murphy[21]Molecular Biology, Princeton University, [2]Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University

Organisms evolve distinct behaviors and physiological responses to adapt to enduring aspects of their environments. However, some shorter-term adaptations can be encoded epigenetically, meaning an ancestor could prime their descendants and kin to better survive in their specific environment. We previously found that upon exposure to pathogen *Pseudomonas aeruginosa*, *C. elegans* employs both transgenerational epigenetic inheritance and horizontal transfer to spread behavioral avoidance of the pathogen to their progeny and community of nearby worms.[1,2] They employ an efficient mechanism that achieves avoidance, transgenerational inheritance, and horizontal transfer through a shared molecular component. This key component is a virus-like particle (VLP) comprised of the protein Cer1.[2] Cer1 is encoded by a retrotransposon and expressed in the germline of adult hermaphrodites, where it packages epigenetic signals in the form of small RNAs.[2] These protected small RNAs then traffic to the neurons of that worm to induce behavioral changes.[2] This germline-to-neuron flow of information is required for avoidance in each subsequent generation before the behavior resets.[2] Additionally, worms secrete these Cer1 VLPs into their environment, where naïve unexposed worms can take up the signal, horizontally spreading protective avoidance.[2] While Cer1 VLPs are the protective vehicle mediating transgenerational and horizontal avoidance, its small RNA cargo encodes the specificity of this behavioral change. Here, we have identified one environmental adaptation spread through Cer1. However, its ability to protect small RNAs for intra- and inter-worm long-range signaling could allow worms to share other important environmental information. Here, we will identify the small RNA cargo of Cer1 VLPs from worms exposed to *P. aeruginosa* to (1) give insights into the neuronal changes associated with *P. aeruginosa* avoidance and (2) identify the small RNA responsible for horizontal transfer of avoidance, and (3) identify small RNA cargo of Cer1 VLPs from unexposed worms to give insights Cer1's potential to transmit other epigenetic changes between worms. Through this work, we will uncover the mechanism Cer1 mediated pathogen avoidance and identify novel uses for Cer1 in spreading environmental information and adaptations.

References:

1. Moore and Kaletsky et al., Cell **177**, 1827-1841 (2019).

2. Moore and Kaletsky et al., Cell **184**, 4697-4712 (2021).

632B **Elucidating mechanisms of susceptibility and resistance to distinct Microsporidia species in wild isolates of *C. elegans*** Meng Xiao[1], Yin Wan[2], Calvin Mok[2], Aaron Reinke[21]Molecular Genetics, University of Toronto, [2]University of Toronto

Microsporidia are rapidly emerging opportunistic infectious pathogens that infect humans and most other organisms including agriculturally important species such as honeybees and fish. Several parasitic microsporidian species have been isolated in wild strains of *C. elegans*. Using PhenoMIP, a multiplexed sequencing-based screen for measuring phenotypes in *C. elegans*, 22 wild strains were tested against four microsporidian species. We identified two strains, JU1400 and MY1, which are sensitive to an epidermal-infecting species, yet resistant to an intestinal-infecting species. Complementation tests between JU1400 and MY1 suggests these strains share variants in the same genes that are responsible for sensitivity and resistance. Using genetic mapping, we identified four distinct loci which may be responsible for these differential phenotypes. Generation of introgressed lines narrowed critical region of the sensitivity phenotype to 750kb on the left arm of Chromosome I. Generation of introgressed lines for the resistance phenotype shows that genes for resistance reside on chromosomes II, V, and X. We are currently testing genes with shared variants in MY1 and JU1400 to identify the causative genes responsible for their susceptibility phenotype. Concurrently, we plan on performing a forward genetic screen to identify genes responsible for JU1400 and MY1 resistance phenotype. Finally, we aim to expand on the PhenoMIP assay to examine a greater number of wild isolates to identify additional strains with variations in fitness. With a larger panel of wild strains, causative variants can be identified through genome-wide-association

studies. Overall, we will identify key molecular mechanisms in host-pathogen interactions between *C. elegans* and microsporidia that serves as a model to understand these types of infections in humans.

633B **Post-transcriptional regulation of the pro-apoptotic BH3-only gene *egl-1*** Yanwen Jiang, Barbara Conradt Cell and Developmental Biology, University College London

Programmed cell death occurs in a highly reproducible pattern during *C. elegans* development and is dependent on the central cell death pathway, whose most upstream component is the pro-apoptotic BH3-only gene *egl-1*. Unlike the other components of the cell death pathway (i.e. *ced-9* Bcl-2, *ced-4* Apaf-1, and *ced-3* caspase), which are broadly expressed, *egl-1* is predominantly expressed in 'cell death lineages'. Our lab previously demonstrated that *egl-1* transcription is initiated in mothers of cells programmed to die. After mother cell division, the number of *egl-1* transcripts increases in the smaller daughter cell that is programmed to die and decreases in the larger daughter cell that survives. In addition to the control of *egl-1* expression at the transcriptional level, regulation at the post-transcriptional level (for example through *mir-35* family microRNAs) has been shown to play a crucial role in the control of *egl-1* expression. The present study demonstrates that several elements, such as the 3' terminal element (TPTE) in the *egl-1* 3' UTR, contribute to the repression of *egl-1* expression. Furthermore, we screened 660 genes predicted to encode RNA binding proteins (RBP) and identified several candidates that may repress or promote *egl-1* expression. RNAi knockdown or loss of function mutations of candidate genes, such as *swsn-7*, cause apoptosis-related phenotypes. We are currently determining the effects of these candidates on *egl-1* mRNA stability and translation using various tools. We are also performing immunoprecipitation experiments to determine whether these candidates directly interact with *egl-1* mRNA. Overall, this study indicates that *cis*-acting elements and *trans*-acting factors of the *egl-1* 3' UTR are involved in the control of *egl-1* expression in cell death lineages and hence contribute to the highly reproducible pattern of cell death during *C. elegans* development.

634B **ICL-1 suppresses the pathogenicity of mitochondrial DNA damage** Chai Chee Ng, Chuan-Yang Dai, Steven ZurynThe University of Queensland

Mutation and molecular damage to the mitochondrial genome (mitochondrial DNA, mtDNA) can cause a range of devastating heritable mitochondrial diseases and common aged-associated disorders. We performed genome-wide screens to identify factors that suppress cellular defects caused by mtDNA double-strand breaks (mtDSBs) in the muscle cells of *Caenorhabditis elegans* and discovered a gain-of-function mutation in the bifunctional metabolic enzyme isocitrate lyase (ICL-1^{T848I}) that stabilizes the enzyme during mitochondrial stress and improves muscle cell function by more than 5-fold. ICL-1^{T848I} acts cell autonomously within damaged mitochondria to catalyze the glyoxylate cycle, shortcuting the tricarboxylic acid (TCA) cycle and thereby improving cellular NAD^+/NADH redox balance during respiratory chain failure. ICL-1^{T848I}-mediated protection against mtDNA damage requires the activities of the cytoprotective factors PARPs and sirtuins that use NAD^+ as a substrate for their roles in DNA damage signaling. Engineering the glyoxylate cycle into mammalian cells through ICL-1^{T848I} expression significantly alleviated hypoxic-induced cell death, suggesting a metabolic strategy to shield cells from severe mitochondrial dysfunction. Together, our results imply that ICL-1^{T848I} rewires core metabolism to preserve NAD^+ for cytoprotective functions during mitochondrial stress.

635B **Determining the cis-regulatory context for HLH-25 mediated repression** Mecca Q Baker Biology, James Madison University

The REF-1 family of basic-Helix-Loop-Helix transcription factors in Caenorhabditis elegans regulates embryonic development, via both Notch-dependent and Notch-independent mechanisms. The REF-1 family member HLH-25 is an ortholog of the mammalian Hairy/Enhancer of Split (HES) protein HES1, which is known to regulate cell proliferation, growth, and differentiation. Previous studies have identified the HLH-25 transcriptional regulatory network, as well as potential regulatory sites that are recognized by the protein. However, little is known about the cis-regulatory context for HLH-25 mediated repression during embryogenesis. We are using chromatin immunoprecipitation sequencing (ChIP-seq) to determine whether HLH-25, and HES proteins in general, act distally or proximally to repress transcription. In preparation for ChIP-seq, we have developed a strain that produces 6X-HIS HLH-25, and that does not express the duplicate gene hlh-27. The data generated through CHIP-seq, in combination with the genome-wide RNA sequencing data will provide insight into whether HLH-25 is a long or short-range repressor of transcription.

636B **Hedgehog-related signaling regulates the formation of starvation-induced gonad abnormalities downstream of Insulin/IGF-1 signaling in *Caenorhabditis elegans*** Ivan B Falsztyn, James Jordan, Ryan Baugh Duke University

When *Caenorhabditis elegans* embryos hatch in the absence of food they undergo L1 arrest. Although arrested L1 larvae can survive for weeks and grow to fertile adults when introduced to food, many animals that experience extended L1 arrest develop gonad abnormalities. These primarily appear as masses of undifferentiated teratoma-like tissue in the uterus and germ-cell tumors in the proximal gonad. Attenuating insulin/insulin-like growth factor signaling by disrupting *daf-2*, the sole known insulin/IGF receptor, suppresses the formation of reproductive abnormalities (Jordan 2019). Previous work showed that early life

starvation and IIS converge on lipid metabolism and Wnt signaling, which promote formation of gonad abnormalities (Jordan 2022, Shaul 2022). In addition, over half of all Hh-related genes are up-regulated in adults following extended L1 starvation and down-regulated following recovery on *daf-2* RNAi. However, the roles and relationships among these pathways downstream of IIS are not understood. RNAi screening of differentially expressed Hh-related genes following extended L1 arrest identified several putative ligands and receptors as mediators of starvation-induced abnormalities. Analysis of fluorescent reporters reveals that Hh-related genes are expressed primarily in the hypodermis and intestine of developing larvae. Furthermore, soma but not germline-specific RNAi against Hh-related genes suppresses abnormalities suggesting a somatic site of action. Further testing through tissue-specific rescue will reveal whether expression in these tissues is sufficient to restore a WT phenotype. Epistasis analysis and mRNA sequencing after recovery on RNAi targeting key lipid metabolism, Wnt, and Hh-related signaling genes followed by meta-analysis and functional characterization of differentially expressed genes will provide further insight into interactions among these pathways. Our current findings shed light on the role of the poorly understood expanded *C. elegan*s Hh-related signaling pathway in mediating a unique example of early-life stress leading to adult pathology.

637B **Dissecting the role of RNT-1 in epidermal stem cell patterning in *C. elegans*** Amanda Lin, Mark Hintze, Dimitris Katsanos, Michalis BarkoulasLife Sciences, Imperial College London

Key events during organismal development are regulated by transcription factors (TFs), which are potent molecules that bind to DNA in a sequence-specific manner to regulate transcription. Numerous TFs have been identified to play a role in epidermal stem cell patterning in *C. elegans*. One of them, RNT-1, is a key TF that is involved in promoting symmetric seam cell divisions during the second larval stage of development. RNT-1 is the homolog of human Runx, and mutations in Runx genes can lead to various diseases in humans, such as leukaemias, osteochondrodysplasia, and breast cancer. Yet, how RNT-1/Runx exert their effects on development and disease remains largely unknown because their direct targets are not well understood. The aim of this project is to investigate direct targets of RNT-1 in seam cells via targeted DamID (TaDa). Through our ongoing TaDa experiments, we have selected a number of potential RNT-1 binding events in proximity to genes known to play a role in seam cell development. To dissect the underlying interactions between RNT-1 and its potential targets, we will employ gene expression analysis via single-molecule fluorescence *in situ* hybridization and functional genetic approaches by combining RNAi-mediated knock down of potential targets and CRISPR-genome editing to edit binding sites and assess their significance for target gene expression. Understanding how RNT-1 positively or negatively regulates target genes will help us to establish a more precise epidermal gene network. Importantly, these findings may also have implications for RNT-1-related biology and disease in higher organisms.

638B **Wormbiome: a comprehensive genomic database of the *Caenorhabditis elegans* microbiome** Adrien Assie, Dana Blackburn, Fan Zhang, Buck S Samuel Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine

Caenorhabditis elegans has been around for over half a century, yet important knowledge gaps still exist in its feeding biology and interaction with associated bacteria. *C. elegans* is no stranger to bacteria. In the laboratory, it is fed on *Escherichia coli* OP50. Many pathogenesis studies explore the infection mechanism of various bacteria, such as *Pseudomonas* (PA01 or PA14), *Staphylococcus*, and others. And recently, multiple studies describing the nematode naturally associated microbiota expanded the catalog of nematode-relevant bacteria and propelled *C. elegans* as a new model for host-microbe interactions research. However, microbiomes can have a crucial influence on animal biology. The effect of those associated bacteria is often overlooked, and interest is focused on host phenotype and biology when it could be associated with or explained by the microbiome.

We created WormBiome, a publicly accessible database (http://www.wormbiome.org) designed to help *C. elegans* researchers explore and analyze the genomic information of nematode-related bacteria. The WormBiome database includes 196 publicly available genomes of bacteria commonly used with *C. elegans* or associated with the nematode in the wild. We set up a pipeline using high-quality assemblies to first annotate each microbial genome using multiple tools and functional databases, where one database can rescue the pitfall of another. Then, generate a comprehensive inventory of each genome's metabolic genes and reconstruct their metabolic network *in silico*. Finally, calculate how closely the different genomes are related to each other based on their functional genomic profile and determine their phylogenomic relationships.

The database-associated website is an interactive platform offering various tools that allow researchers to browse, search, or compare interactively specific microbial genetic features directly by name, functional category, or raw sequence using blast. We also provide BiomProfiler, a pipeline that uses our database and 16s rRNA amplicon sequencing data to generate functional microbial profiles and associate host nematode to microbe-specific genetic features. All the data underlying the database is also available for direct download for any user to include in custom analyses or pipelines.

WormBiome is poised to become the central repository for *C. elegans*-related microbial genomes through active and community-driven data and literature curation and development for future website features.

639C **Distinguishing the sex-specific roles of germline small RNA pathways** Mathias S. Renaud, Amanda G. Charlesworth,

Uri Seroussi, Julie M. Claycomb Molecular Genetics, University of Toronto

With diverse roles in essential biological processes including development, genome stability, and fertility, small RNA (sRNA) pathways are key regulators of gene expression. sRNAs direct sequence-specific gene regulation via association with effector Argonaute proteins (AGOs), resulting in degradation, translational inhibition, or licensing of target transcripts. Our lab has systematically characterized the expression patterns and associated sRNA populations of the 19 C. elegans AGOs. 16 of these AGOs are expressed in the gonad, pointing to their importance in gametogenesis, and among these, four AGOs are expressed in the gonad only during spermatogenesis (ALG-3, ALG-4, CSR-1a, & WAGO-10). While sRNA pathways have been shown to be important for sperm mediated fertility in many organisms, including C. elegans, the specific mechanisms by which these pathways regulate sperm differentiation and function remain elusive. Therefore, our goal is to understand how these sRNA pathways contribute to proper sperm development and function in both males and hermaphrodites. By assessing fertility in different combinations of spermatogenesis-specific ago mutants, we have observed stress-induced reductions in fertility that can be rescued by mating to wild-type males, pointing to defects in spermatogenesis. Furthermore, we have also uncovered sex-specific differences in sperm development, number, and function in ago mutant hermaphrodites compared to males that highlight sex-specific differences in spermatogenic gene regulatory programs.

In addition to the four spermatogenesis-specific AGOs, our studies have shown that nine AGOs are expressed in the hermaphrodite gonad during both spermatogenesis and oogenesis. Previously, these AGOs have primarily been studied in the context of the adult hermaphrodite during oogenesis. By studying the targets of these AGOs in hermaphrodites undergoing spermatogenesis, we have shown that WAGO-1, HRDE-1, PPW-1, PPW-2, PRG-1, ALG-5, and RDE-1 have distinct spermatogenesis-specific and oogenesis-specific targets, while CSR-1b and WAGO-4 target a common group of targets during both stages. This stratification of germline AGO targets supposes dual roles for many of these germline-constitutive AGOs in regulating spermatogenesis and oogenesis. Our current efforts are focused on defining the molecular mechanisms by which the spermatogenesis and germline constitutive AGOs contribute to the development and differentiation of fertile sperm.

640C **Mating increases cytosolic protein oxidation in somatic cells in *C. elegans* via H_2O_2 from sperm mitochondria** Yuyan Xu[1], Javier Apfeld[21]Biology, Northeastern University, [2]Northeastern University

The reversible formation of disulfide bonds between cysteine residues plays an important role in the regulation of cytosolic proteins involved in a wide variety of cellular processes associated with aging and aging-related diseases. We set out to study the role of the germline in the regulation of the oxidation of cytosolic proteins in *C. elegans* somatic cells.

We monitored protein oxidation tracked via quantitative ratiometric fluorescence microscopy in live hermaphrodites expressing a genetically encoded biosensor (*roGFP1-R12*) whose oxidation is determined by the glutathione redox couple—a major determinant of cysteine oxidation. We found that germline ablation lowered protein oxidation in the pharyngeal, vulval muscles, and intestine, but did not affect protein oxidation in the PLM sensory neurons. Some of these effects were mediated by the DAF-16/FOXO transcription factor. Thus, signals from the germline normally increased cytosolic protein oxidation in a tissue-specific manner in part via DAF-16/FOXO.

Next, we determined which germline cells regulated protein oxidation in the pharyngeal and vulval muscles, using mutants lacking sperm, oocytes, or both. Sperm regulated protein oxidation in both pharyngeal and vulval muscles while oocytes regulated protein oxidation only in the pharyngeal muscles. Restoring sperm via mating with males increased protein oxidation not only in hermaphrodites lacking self-sperm but also in those lacking all germline cell types. These effects were not due to copulation, seminal fluid, or male pheromone, suggesting that the oxidizing factor is sperm derived.

Using males deficient in the mitochondrial matrix-localized superoxide dismutase SOD-2, we identified a sperm-derived factor that increases protein oxidation in somatic tissues: mitochondrially derived hydrogen peroxide. Our studies suggest that sperm may influence the function of cytosolic proteins in somatic tissues via global changes in the oxidation of cysteine residues.

641C **Understanding the regulatory context and mechanism of HLH-25 mediated transcriptional repression** Dagmawi Mamo, Casonya Johnson Biology, James Madison University

The HES family of transcription factors are primarily repressors of transcription that play critical roles in mammalian embryogenesis by regulating cell differentiation and proliferation. Proper functioning of HES proteins is crucial in humans, as mutations or misexpression of HES1 is associated with lung, pancreas, colon, and other cancers. HES1 may mediate short-range or long-range repression through DNA-binding and or protein-protein interactions with other factors. However, prior genetic studies have not fully elucidated how the context-specific binding of HES1, or its *C. elegans* ortholog HLH-25, promotes the repression of

transcription. Characterization of the cis-regulatory context and mechanism of HLH-25 mediated repression could thus serve as a model for studying the role of HES1 in disease. In the present study, we used an RNA-sequencing based approach to identify differentially expressed genes (DEGs) by comparing the early embryonic transcriptomes of wildtype (N2) and *hlh:25;hlh:27* double knockout animals. Gene ontology analysis of the DEGs demonstrates that HLH-25 regulates genes that are involved in essential cellular roles such as metabolism, DNA-repair, chemosensation, and transmembrane transport. Computational mapping of HLH-25 to transcriptional regulatory elements of its embryonic gene targets shows preferential binding of the TF at cis-regulatory elements located distal to transcription start sites, in a consistent manner across genes with different biological roles. Taken together, our findings suggest that the most likely mechanism for gene-specific repression by HLH-25 is through a distally-bound long-range repressive activity of recruiting histone deacetylases or remodeling chromatin at or near the promoter.

642C **Target-specific requirements for RNA interference can be explained by a single regulatory network** Daphne R Knudsen[1], Pravrutha Raman[1,2], Farida Ettefa[1,3], Laura De Ravin[1], Antony M Jose[11]Cell Biology and Molecular Genetics, University of Maryland, College Park, [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, [3]Institute for Systems Genetics, New York University School of Medicine

Since double-stranded RNA (dsRNA) is effective for silencing a wide variety of genes, all genes are typically considered equivalent targets for such RNA interference (RNAi). Yet, loss of some regulators of RNAi in the nematode *C. elegans* can selectively impair the silencing of some genes, raising the possibility of gene-specific specialization of the RNAi mechanism. Here we dissect the silencing of two somatic genes in detail to show that such selective regulation can be explained by a single network of regulators acting on genes with differences in their RNA metabolism. In this network, the Maelstrom domain-containing protein RDE-10, the intrinsically disordered protein MUT-16, and the Argonaute protein NRDE-3 work together such that any two are required for silencing one gene, but each is singly required for silencing the other gene. While numerous features could distinguish one gene from another, quantitative models suggest that, for the same steady state abundance of mRNA, genes with higher rates of mRNA production are more difficult to knockdown with a single dose of dsRNA and recovery from knockdown can occur if all intermediates of RNA silencing turnover. Consistent with such dissipation of RNA silencing that relies on a single network, (1) animals recover after silencing by a pulse of dsRNA, (2) there is limited production of templates for amplifying small RNAs, and (3) enhancing dsRNA processing can overcome the requirement for the Argonaute NRDE-3. These insights explain selectivity in the requirements for specific regulators without invoking different mechanisms for different sets of genes.

643C ***C. elegans* DOT-1.1 and mammalian DOT1L regulate MYC/Mondo-mediated transcription by promoting the transcription factor degradation cycle on chromatin** Gian Sepulveda, Ekaterina Gushchanskaia, Alexandra Mora-Martin, Ruben Esse, Ainhoa Ceballos, Andrew Emili, Dafne Cardamone, Valentina Perissi, Alla Grishok Biochemistry, Boston University

Our studies of the conserved *C. elegans* DOT-1.1 revealed a functional connection to a transcription factor homologous to the MYC superfamily proteins, Myc- and Mondo-like 1 (MML-1). We found overlapping sets of genes similarly upregulated and downregulated in mml-1 and dot-1.1 mutants and a co-localization of DOT-1.1 and MML-1 at target genes. Furthermore, double mutants of dot-1.1 and mml-1 show similar perturbation of MML-1 target gene expression as the single mutants. Surprisingly, promoter occupancy of MML-1 (via ChIP-qPCR) was elevated in dot-1.1 mutant worms, despite changes in gene expression mimicking those of mml-1(-) (i.e. the lack of MML-1 at the promoters). We expand this cooperation of DOT-1.1and MML-1 into mammalian triple negative breast cancer cells, wherein both acute (siRNA treatment) and permanent (CRISPR/Cas9-based) knock-down of DOT1L led to reduced expression of c-MYC target genes (assessed by RNA- seq, proteomics, and RT-qPCR) accompanied by elevated occupancy of c-MYC at their promoters. Nuclear proteasome plays an important role in removing transcription factors from chromatin. In the case of c-MYC, it was shown that its timely removal by the ubiquitin- proteasome system (UPS) was critical for the target gene activation. Indeed, we recapitulated reduced expression of c-MYC/DOT1L target genes upon proteasome inhibitor treatment. However, the inhibitor effect was dampened in dot1l (+/-) cells already deficient in c-MYC target gene expression indicating that DOT1L and nuclear proteasome function in the same pathway. Because inhibition of the methyltransferase activity of DOT1L failed to induce enhanced c-MYC occupancy at the promoters, we believe that DOT1L regulates c-MYC turnover in a non-canonical manner. Importantly, our bioinformatic analyses of RNA-seq and proteomics data obtained with dot1l (+/-) cells indicate perturbation of Mondo B and other transcription factor networks, in addition to the c-MYC network (top scoring). This is consistent with the effect of dot-1.1 mutation on MML-1 in *C. elegans*. Earlier, we have described elevated stability and enhanced promoter occupancy of the MML-1(ok849) mutant protein containing a large internal deletion. mml-1(ok849) behaves as a loss-of-function allele according to multiple publications, and we now show that MML-1(ok849) is less susceptible to regulation by the UPS system, thus proving the functional importance of transcription factor turnover on chromatin from nematodes to humans.

644C **Sexually dimorphic germ granule structure affects *C. elegans* fertility** Acadia L DiNardo, Hannah R Wilson, Rachael M Giersch, Nicole A Kurhanewicz, Diana E Libuda Biology, University of Oregon

Small RNA pathways utilize Argonaute proteins to monitor and protect the germline genome by regulating gene expression during germ cell development. Argonaute proteins process small RNAs in germ granules, which are liquid-like compartments that form adjacent to nuclear pore complexes. In *Caenorhabditis elegans,* some Argonaute proteins (WAGOs) regulate distinct RNA targets in a sex-specific manner during egg and sperm development. How the WAGOs regulate gene expression in a sexually dimorphic manner is largely unknown. Here we show that the intrinsically disordered N-terminus of three Argonaute proteins (WAGO-1, WAGO-3, and WAGO-4) confers sexually dimorphic functions in germ cells by regulating the stability, composition, and structure of phase separated subcompartments of germ granules. During meiotic prophase I progression, WAGOs-1/3/4 and the 21U-piRNA pathway Argonaute PRG-1 display distinct and dynamic localization patterns relative to germ granule structural proteins that define the P-granule (PGL-1) and Z-granule (ZNFX-1) subcompartments. While WAGO-4 associates with both the P- and Z-granule during oogenesis, WAGO-4 does not associate with either of these germ granule subcompartments during spermatogenesis. WAGOs-1/3 co-localize and interact with the P- and Z-granules more frequently during spermatogenesis than in oogenesis. Moreover, the size of WAGOs-1/3/4 and PRG-1 foci are significantly different between spermatogenesis and oogenesis and are dependent upon the dosage of the Z-granule structural protein, ZNFX-1. Finally, we find that disruption of the N-terminus of WAGOs-1/3/4 causes both spermatocyte-specific infertility and spermatocyte-specific loss of the P granule in late prophase I. These results suggest the N-terminus of WAGOs-1/3/4 has spermatocyte-specific functions for phase separating the P-granule subcompartment within the germ granule to promote male fertility. Taken together, our study indicates Argonaute proteins interact with PGL-1, ZNFX-1, and other binding partners critical for germ granule structure and function to enable sex-specific gene expression during germ cell development.

645C **A SID-1-dependent gene within a retrotransposon enhances heritable RNA silencing** Aishwarya Sathya, Nathan Shugarts, Andrew Yi, Antony JoseUniversity of Maryland

RNAs in circulation carry sequence-specific regulatory information between cells in animal, plant, and host-pathogen systems. Such extracellular RNAs can function across generational boundaries in *C. elegans*. Double-stranded RNA (dsRNA) from neurons or the body cavity can enter the cytosol through the dsRNA importer SID-1 and silence genes of matching sequence in the germline and in progeny. Here we show that such SID-1-dependent regulation reduces heritable RNA silencing by downregulating a gene located within a retrotransposon. Using RNA-seq of wild-type, *sid-1(-)* mutant, and reverted *sid-1(+)* animals, we identified a ***s**id-1*-**d**ependent **g**ene *sdg-1*, which is located within a copy of the Cer9 retrotransposon. Changes in SDG-1 expression upon loss of SID-1 can last for more than 100 generations after SID-1 is restored. Perturbations of RNA-mediated regulation within the germline result in opposite effects on *sid-1* and *sdg-1*. Animals that lack SID-1 or that overexpress SDG-1 both show enhanced initiation of heritable RNA silencing upon mating. Furthermore, the SDG-1 protein is abundant within the germline and colocalizes with perinuclear condensates called Z granules, which are required for heritable RNA silencing. Since such silencing targets retrotransposons, these results suggest an auto-inhibitory loop that limits silencing of the *sdg-1*-containing retrotransposon. Intriguingly, SDG-1 (encoded by F07B7.2/W09B7.2) has two other paralogs ZK262.8 and C03A7.2, both of which are encoded by genes located within Cer8 retrotransposon sequences. Furthermore, loss of ZK262.8 has been reported to be synthetic lethal with loss of the miRNA-associated Argonaute ALG-2. Therefore, we speculate that inclusion of genes that regulate RNA silencing enables some retrotransposons and retrotransposon-derived sequences to persist over evolutionary time.

646C **The evolution of sexually dimorphic morphogenesis** Raya Jallad[1], Alyssa Woronik[2], Karin Kiontke[1], Yash Patel[1], David Fitch[11]New York University, [2]Sacred Heart University

Differences between sexes are common in nature. However, how the development of sexual dimorphism is regulated is insufficiently known. As a model system to better understand the gene regulatory network (GRN) responsible for sexually dimorphic morphogenesis and its evolution, we study the 4 tail tip cells of *Caenorhabditis elegans*. During the last larval stage, in males only, these cells undergo a process known as Tail Tip Morphogenesis (TTM), resulting in round tail tips in males (hermaphrodites retain the pointed tips of the larvae). The DMRT transcription factor DMD-3 is required and sufficient for TTM in *C. elegans* and is hypothesized to be at the center of a bow-tie GRN (Mason et al. 2008, Nelson et al. 2011). This is consistent with the fact that such DMD factors are conserved in the regulation of male-specific traits in many animals. TTM evolved multiple times independently in related nematode species, but whether or not the functional role of DMD-3 in TTM is conserved remains unknown. One hypothesis for convergent evolution (aka the “hotspot” hypothesis) predicts that the architecture of GRNs biases evolution, such that the same genes/modules are co-opted when similar traits evolve repeatedly. To test this hypothesis for the convergent evolution of TTM, we aim to compare TTM transcriptome profiles among several rhabditid species. As a first step in this comparison, we are characterizing transcriptome dynamics in *C. elegans* males, hermaphrodites, and mutants of DMD genes using tail-tip-specific RNA-seq at several time points during the last larval stage, when TTM occurs in males. So far, we have established the expression profiles of individual genes in WT and *dmd-3(-)* males and WT hermaphrodites. We are also sorting genes into different categories of gene expression dynamic (e.g. static, upregulated, downregulated, etc.). By comparing such profiles between species where TTM was conserved, independently gained, lost, or ancestrally absent, we will identify which genes/modules have been conserved or not and test the «hotspot» hypothesis for sexually dimorphic morphogenesis.

647C **Single nucleus RNA sequencing of neurons in Caenorhabditis elegans** Jonathan St. Ange, Rachel Kaletsky, Morgan Stevenson, Coleen Murphy Princeton University

Until recently, transcriptome analysis in C. elegans was limited to bulk RNA sequencing assays that measure the average expression across individual tissues or the whole worm. Bulk RNA sequencing may fail to detect rare cell-type specific gene expression signatures, and single-cell and single-nucleus RNA-seq approaches in C. elegans aim to overcome these limitations. However, the unique composition of brain tissue, including distally localized mRNAs in fragile dendrites and axons, which may be lost during cell dissociation, as well as the limited abundance of neurons relative to cells and nuclei in the whole adult animal, suggest that unique approaches are needed accurately assess individual neuron-specific gene expression in adults. Borrowing from the highly successful single-nucleus RNA-seq methods used to assay mammalian neurons sorted from brain tissue, we adapted these methods to label and FACS sort C. elegans neuron nuclei for single nucleus RNA sequencing. We find that neuron single-nuc RNA-seq allows robust single neuron resolution and permits the identification of differentially expressed genes using genetic mutants. Single-nucleus RNA-sequencing of C. elegans neurons has the potential to uncover how gene expression changes in individual neurons contribute to complex behaviors and phenotypes.

648C **The germline RNAi pathway requires RNA helicase A for effective siRNA production** Olivia Gaylord, Jordan Brown, Heng-Chi LeeMolecular Genetics and Cell Biology, University of Chicago

In the *C. elegans* germline, both endogenous and foreign RNAs are regulated by a small RNA network consisting of multiple Argonaute effectors that bind distinct classes of small RNAs. These small RNA pathways share an overlapping set of core components, raising the question of how the specificity and function of each pathway is determined. Since RNA Helicase A (RHA-1) is required for RNAi in germ cells but not somatic cells, we hypothesized RHA-1 is not a core RNAi component, but rather a regulator of RNAi in germ cells.

In the RNAi pathway, Dicer processes dsRNA into siRNAs, which trigger the production of secondary 22G-RNAs, the effectors of gene silencing. To gain molecular insight on RHA-1 function in RNAi, we examined the production of siRNAs and secondary 22G-RNAs in worms fed bacteria expressing *cdk-1* dsRNA. As expected, wild-type worms produce *cdk-1* targeting siRNAs predominantly 23 nucleotides in length and abundant secondary 22G-RNAs. Strikingly, *rha-1* mutant worms produce over 5-fold more *cdk-1* targeting siRNAs of mostly aberrant size ranging from 20 to 35 nucleotides in length. Although *rha-1* mutants exhibit abundant abnormal length siRNAs, the secondary 22G-RNAs are produced to similar levels as wild-type, yet these 22G-RNAs fail to induce gene silencing. We hypothesized that the overabundance of abnormal size siRNAs may overload the RNAi pathway components, decreasing efficiency of 22G-RNA mediated gene silencing in *rha-1* mutants. Therefore, we examined whether deletion of other germline small RNA pathways could free up shared components of the RNAi pathway and thus rescue the RNAi defect in *rha-1* mutants. Indeed, we find that either loss of germline 26G-RNA pathway factors *rrf-3* or *gtsf-1*, or germline piRNA pathway Argonaute *prg-1* strongly rescues the germline RNAi defect of *rha-1* mutants. Although not the only possible model, we propose RHA-1 functions as a critical regulator of the RNAi pathway in germ cells by promoting the turnover of abnormal length siRNAs. Overall, our results highlight the intricacies of the small RNA regulatory network and need for additional regulators in germ cells to ensure animal development and fertility.

649C **Investigating how the HPV E7 oncoprotein disrupts the function of the DREAM transcriptional repressor complex** Emily Washeleski[1], Ryleigh Parsons[1], Paul D Goetsch[21]Michigan Technological University, [2]Biological Sciences, Michigan Technological University

E7, a small protein encoded by the Human Papilloma virus (HPV), plays a large role in the oncogenic progression of infected cells. Approximately 5% of cancer cases worldwide develop from HPV infections. Although there is an available vaccine that helps prevent infection from the most common oncogenic HPVs, there remains no treatment to prevent oncogenesis if a patient does get infected. Oncogenic HPV E7 proteins cause cancer via disrupting the transcriptional regulation of the cell cycle by inhibiting the function of the 3 mammalian pocket proteins, the Retinoblastoma protein (pRb), p107, and p130. Since HPV E7 interacts indiscriminately with the pocket proteins through their “LxCxE” binding cleft, the viral oncoprotein impairs p107 and p130 from forming the DREAM transcriptional repressor complex and pRb’s tumor suppressor activity as a major checkpoint inhibitor of early cell cycle progression. The goal of this project is to better understand how the HPV E7 oncoprotein disrupts DREAM complex function by establishing HPV E7 transgenic lines in *Caenorhabditis elegans* using CRISPR/Cas9 mediated genome editing. Because the DREAM complex subunits are highly conserved, the sole *C. elegans* pocket protein homolog *lin-35* is likely a candidate for binding by HPV E7 proteins. With over 200 strains of HPV classified, oncogenic HPV E7 proteins share a conserved LxCxE binding motif identical to the interaction motif required for LIN-52, another DREAM subunit, to bind to LIN-35 and form the *C. elegans* DREAM complex. I hypothesize that oncogenic HPV E7 proteins will compete against LIN-52 for association with the LIN-35 pocket protein, inhibiting DREAM complex formation and function. In contrast, I expect that non-oncogenic HPV E7 proteins will not disrupt DREAM formation. In *C. elegans*, loss of DREAM complex activity is readily observed via high penetrance

of a synthetic multivulval (synMuv) phenotype when combined with SynMuv A class gene mutants. Once functional transgenic worm strains are established, I will develop a screen of small molecules and compounds with known, as well as modeled, activity against HPV E7 proteins. Ultimately, we aim to use this system for rapid screening of *in vivo* of compounds capable of disrupting the HPV E7 mediated oncogenic process.

650C **Inheritance of *Sid-1* epigenetic silencing is modulated by bacterial diet and growth temperature** Nicole M Bush, Craig P HunterMolecular and Cellular Biology, Harvard University

Introduction of an extrachromosomal *sid-1* promoter transgene array (*Psid-1*) silences endogenous *Sid-1* (Minkina and Hunter, 2016). Silencing occurs within both the germline and soma and is incredibly stable, persisting at 100% transmission for up to 13 generations after the array has been lost. Germline silencing requires nuclear RNAi proteins *hrde-1*, *nrde-2*, *and mut-2,* and partially requires *prg-1* for initiation of silencing. Small RNAs transmit silencing, which is paramutagenic and transfers through only the maternal germline.

When *Psid-1* array worms are reared for several generations on HB101 or HT115, but not OP50, transmission of *sid-1* silencing to non-array carrying progeny is reduced. This process is temperature-dependent; higher growth temperatures result in a complete absence of heritable silencing in non-array progeny. The weak RNAi food *dpy-11* requires parental germline *sid-1* function (expression) to produce Dpy progeny. Use of this assay reveals a vibrant picture of the sensitivity of *Sid-1* epigenetic inheritance to environmental conditions. Analysis of individual L4 parents reveals how administration of silencing from parent to progeny differs based on both temperature and food. Furthermore, additional tests with seemingly minimal changes to bacterial and plate preparation reveal that silencing inheritance is incredibly sensitive to subtle changes in often overlooked aspects of worm propagation techniques. Work with the *C. elegans* microbiome confirms that these foods also induce changes in *Sid-1* silencing, which mirror the growth condition effects observed for laboratory *E. coli* strains (Dirksen et al, 2020). We are using RNA sequencing, mass spectrometry and mutant analysis to determine how growth conditions and epigenetic inheritance are interconnected.

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651C **Functional partners SET-26 and HCF-1 are opposed by HDA-1 in chromatin and lifespan regulation** Felicity J Emerson[1], Caitlin Chiu[1], Laura Y Lin[1], Christian G Riedel[2], Ming Zhu[3], Siu Sylvia Lee[11]Department of Molecular Biology and Genetics, Cornell University, [2]Department of Biosciences and Nutrition, Karolinska Institute, [3]National Institute of Biological Sciences

SET-26 and HCF-1 are two highly conserved chromatin factors that play a key role in longevity determination in *C. elegans*. We previously showed that SET-26 and HCF-1 both act in somatic cells to modulate longevity through the transcription factor DAF-16. Here we investigate the relationship between SET-26 and HCF-1 at chromatin and in longevity modulation. We show that SET-26 and HCF-1 operate in the same pathway to modulate longevity and that the histone deacetylase HDA-1 plays an antagonizing role, as the extended longevity of the *set-26* and *hcf-1* mutants requires functional *hda-1*. Mechanistically, SET-26 is recruited to chromatin via its PHD domain, which binds the active mark H3K4me3 when flanked by acetylation. HCF-1 depends on SET-26 to be recruited to chromatin, possibly through the highly conserved HCF-1 binding motif of SET-26. SET-26 and HCF-1 co-occupy the promoters of many genes and appear to stabilize each other on a subset of those genes, particularly those involved in mitochondrial metabolism. HDA-1 is also localized to many of the SET-26 and HCF-1 binding sites across the genome, but its recruitment to chromatin does not depend on SET-26 nor HCF-1. Gene expression profiling reveals that SET-26 and HCF-1 affect gene expression similarly, whereas HDA-1 often has opposing effects, particularly on mitochondrial metabolism genes, consistent with its antagonistic role in longevity determination. Our findings suggest that SET-26, HCF-1, and HDA-1 comprise a mechanism to fine-tune gene expression and longevity and likely have important implications for the mechanistic understanding of how these factors function in diverse organisms.

652C **Uncovering germ granule proteomes using TurboID** Sebastian Fuentes, Adam Sundby, Uri Seroussi, Julie ClaycombMolecular Genetics, University of Toronto

Fertility is a key biological process that ensures the survival of species. There are many levels of control over gene expression that ensure fertility, and small RNA (sRNA) pathways have emerged as critical regulators in gametogenesis. sRNAs are a subset

of non-protein coding RNAs 18-26 nucleotides long that associate with Argonaute proteins (AGOs). sRNAs guide AGOs to target transcripts by sequence complementarity, leading to regulation through a variety of mechanisms such as mRNA degradation, chromatin modification, and translational repression. Remarkably, *C. elegans* possesses 19 functional AGOs, with 16 of 19 AGOs being expressed in the germline, and 8 being associated with phase separated organelles known as germ granules.

C. elegans possesses four types of germ granules: P granules, Z granules, Mutator Foci, and SIMR Foci, which are key for optimal fertility. Microscopy and genetic approaches have identified varying numbers of the constituents of germ granules, and the field has found that different sets of sRNA pathway components including AGOs, RNA binding proteins, and RNA dependent RNA polymerases reside in different granules. However, while we have learned a great deal about germ granules over the past 30 years, we have yet to comprehensively identify, categorize, and understand the functions of all germ granule proteins. New technologies including CRISPR-Cas9 genome editing and *in vivo* proteomic methods, such as TurboID/BioID will enable us to tackle this challenge.

TurboID is an *in vivo* proximity labeling approach that enables the biotinylation of proteins within a 10nm radius. The biotinylated interacting proteins can then be isolated using streptavidin pull-down and identified using mass-spectrometry (MS). We are using TurboID to systematically identify the protein components of germ granules. We first focused on Z granules, which are involved in RNAi inheritance and fertility, and have only a handful of known components. We fused TurboID to known Z-granule proteins ZNFX-1 and WAGO-4 and performed AP-MS on young adult hermaphrodites. Our results reveal a variety of new putative Z granule proteins. We are now examining the localization and phenotypes of these new putative germ granule proteins, and expanding our TurboID studies to all germ granule AGOs. Ultimately we aim to bootstrap our way through each germ granule proteome in an effort to map out the pathways and elucidate the molecular mechanisms housed within these key organelles.

653C **Evidence for multiple forms of heritable RNA silencing** Mary Chey[1], Victoria Murphy[1], Pravrutha Raman[1], Farida Ettefa[1], Rui Yin[2], Antony Jose[11]Cell Biology Molecular Genetics, University of Maryland, [2]COMPUTATIONAL BIOLOGY, BIOINFORMATICS, AND GENOMICS, University of Maryland

Changes in the expression of a gene can be maintained for hundreds of generations without causal mutations in DNA. Such heritable epigenetic changes have been observed in the nematode *C. elegans*, where the transgenerational silencing of a gene can be correlated with changes in small RNA and chromatin. Here we demonstrate that multiple mechanisms regulate transgenerational silencing of foreign genes and reveal how their expression perturbs RNA regulation within the germline to facilitate heritable epigenetic changes. One cistron of a two-gene operon can be selectively silenced for many generations independent of the nuclear Argonaute HRDE-1, revealing a mechanism that operates on spliced transcripts without relying on co-transcriptional or chromatin-mediated silencing. Reverting *hrde-1(-)* mutants to wild type can result in siblings with or without heritable expression of a foreign gene, generating both expressed and silenced versions of the gene with the same *cis*-regulatory sequences. Yet, different *cis*-regulatory sequences can also influence the susceptibility of the gene to transgenerational silencing. The presence of mRNA-derived templates with poly-UG tails that are used for the amplification of small RNAs have been proposed as a hallmark of gene silencing. Yet, these pUG RNAs are detectable in animals with or without expression of the gene, suggesting that their presence is not sufficient for observing silencing. Animals with the foreign gene show a reduction in mRNAs from multiple germline-enriched genes that encode for potential regulators of RNA silencing, which could explain the susceptibility of such animals to transgenerational gene silencing. Together these results suggest that expression of a foreign gene can disrupt RNA regulation in the germline to alter multiple mechanisms that maintain heritable epigenetic changes.

654C **Assessment of annotation transfer approaches for single-cell RNA-seq analysis** Rupa Khanal[1], Christopher R L Large[1], LaDeana Hillier[2], Chau Hyunh[2], Priya Sivaramakrishnan[1], Felicia Peng[1], Qin Zhu[3], Erik Nordgreen[1], Jean Rosario[1], Junhyong Kim[1], Robert H Waterston[2], John I Murray[11]University of Pennsylvania, [2]University of Washington, [3]University of California, San Francisco

Comparing scRNA-seq datasets can reveal how gene expression patterns change across different tissues, stages, genotypes, conditions and species for diverse cell types. However, identification of the equivalent cell type labels in each dataset is essential for such comparisons and manual annotation based on marker gene expression can be prohibitively time-consuming and inefficient for large datasets. Automated annotation transfer methods can identify homologous cell types and transfer labels of interest from an annotated reference to query datasets. However, a better framework for selecting the best approach for annotation transfer depending on the datasets being used is needed.

The highly conserved embryonic lineage of Caenorhabditis species allows benchmarking annotation transfer methods at single cell resolution across species, making them a powerful system for testing the performance of annotation transfer. In this study, we evaluate the performance of current annotation transfer methods using manually annotated single-cell RNA-seq datasets of developing embryos of C. briggsae and C. elegans. We used these datasets to evaluate the commonly used CCA and PCA

algorithms in Seurat for their performance in transferring progenitor lineage identity to cells of the same or different species. We quantified the extent of matches of predicted labels with manual labels for each transfer. We further assessed the robustness of each algorithm by performing cross-validation and analyzing their stability across different datasets. Additionally, we explored the impact of variables like feature selection methods, cell type complexity and distinctiveness, and evolutionary distance between the species being compared on annotation transfer performance. Our results demonstrate that the performance of current annotation transfer methods varies significantly depending on the dataset, and is significantly affected by the number and types of genes used for the transfer. Cell type complexity, such as the number of cell type-specific genes and the number of cells sampled per cell type, also leads to differing performance for different cell types. Additionally, annotation transfer performance across species is lower than within species.

Overall, we provide valuable insights into the performance of current annotation transfer methods, highlighting the need for careful consideration of various factors when selecting the appropriate method for a specific problem and dataset.These insights can ultimately improve the accuracy and reliability of cell type identification in these analyses.

655C **Worms use both their mouth and their nose to chemotax away from hydrogen peroxide** Yuyan Xu[1], Maedeh Seyedolmohadesin[2], Alyson Fulton[2], Eyob Gebeyaw[2], Meagan Duncan[2], Katerina Gusarova[2], Matea Zelich[3], Aishwarya Sood[2], Olivia Liu[2], Vivek Venkatachalam[2], Javier Apfeld[21]Biology, Northeastern University, [2]Northeastern University, [3]Harvey Mudd College

One of the most common lethal threats that nematodes encounter is hydrogen peroxide (H_2O_2), which is produced by a wide variety of microorganisms. In this microbial battlefield, how do worms find an environment safe from the threat of H_2O_2?

Using calcium imaging, we identified at least six classes of amphid neurons, including ASK, ASJ, AWC, AFD, ADL, and URX, and eight classes of pharyngeal neurons, including I2, I3, I4, I5, M1, M3, M5, and MI, that exhibit distinct concentration-dependent responses to environmental H_2O_2. Using a combination of molecular-genetic and cell ablation studies, we found that both H_2O_2-sensing pharyngeal and amphid neurons play non-redundant roles in promoting chemotaxis away from H_2O_2. The I2 neurons in the pharynx respond to micromolar concentrations of H_2O_2 to promote H2O2 avoidance via a GUR-3 gustatory receptor-dependent mechanism. The ASK neurons in the amphid respond to millimolar H_2O_2 concentrations to promote H2O2 avoidance likely via a process that relies on DAF-11receptor guanylate cyclases and TAX-2/TAX-4 cyclic GMP-gated channels. Furthermore, other amphid neurons that do not respond to H_2O_2, including ASH and AWB, are also required for efficient chemotaxis away from H_2O_2. We are identifying signals that each of these sensory neurons uses to communicate with interneurons to promote H_2O_2 avoidance and the receptors that these interneurons use to respond to those signals.

In summary, we identify roles for pharyngeal and amphid sensory neurons in the perception and response to hydrogen peroxide. While the role of amphid neurons in chemosensation has long been recognized, the role of pharyngeal neurons has not been explored systematically. We propose that, because of their physical location at the site of food ingestion, pharyngeal neurons in *C. elegans* may play a general role in taste perception similar to that of sensory neurons in the mouth in mammals.

656C **Metabolic regulation of the RNA methylome in *C. elegans*** Katarzyna Hencel[1], Aykut Shen[1], Evelyne Deery[2], Gulcin Baykal[3], Aduragbemi Adesina[1], Martin Warren[4], Alper Akay[11]University of East Anglia, [2]University of Kent, [3]Yildiz Technical University, [4]Quadram Institute

RNA modifications are essential for multiple gene regulatory processes, including RNA stability, degradation, and splicing. Research on individual RNA modifications has led to many important discoveries in recent years. However, we know very little about how RNA modifications are regulated in cells and organisms. One of the most abundant modifications on RNA is methylations. Methyltransferase enzymes use S-adenosylmethionine (SAM) to transfer a methyl group to their targets, and cells use methionine to synthesise SAM. Following methylation reactions, SAM is converted to S-adenosyl-L-homocysteine (SAH), followed by conversion to homocysteine which is then methylated to regenerate methionine by the methionine cycle. Therefore, the methionine cycle regulates cellular SAM levels and potentially could regulate all cellular methylations. To understand how RNA methylome responds to changing SAM levels, we used previously established genetic and dietary methods to inhibit the methionine cycle (MacNeil *et al*., 2013, Bito *et al*., 2013). We will present our data on how perturbing the methionine cycle leads to changes in RNA methylation levels and gene expression changes during C. *elegans* development.

657C **Histone methyl-lysine readers CEC-3 and CEC-6 promote germ granule integrity and small RNA regulation** Tammy L Lee, Chengyin Li, Phoebe AW Bhagoutie, Aly Muhammad Ladak, Reta Aram, Elizabeth Yan, Victor Lao, Arneet L SaltzmanCell and Systems Biology, University of Toronto

Small RNA and chromatin regulation pathways cooperate to safeguard germline immortality and the inheritance of genetic information. Importantly, many factors involved in small RNA-mediated gene regulation reside in evolutionarily conserved peri-

nuclear germ granules. We previously found that two *C. elegans*chromodomain proteins, *C. elegans* Chromodomain (CEC)-3 and CEC-6 recognize heterochromatin-associated histone H3K9 and H3K27 methylation *in vitro*. *cec-3; cec-6* mutant animals show a progressive sterility or 'mortal germline' phenotype, but the underlying cause of this fertility defect is unknown. Live imaging analysis of GFP-tagged germ granule components PGL-1 and DEPS-1 reveals that germ granule formation is disrupted in *cec-3; cec-6* mutant adult germlines. Furthermore, progressive loss of germ granule integrity was accompanied by defects in meiotic prophase progression as *cec-3; cec-6* lines approach sterility. Our transcriptome analysis using RNA-seq and RT-qPCR suggest that a subset of repetitive elements, transposons, germ granule components, and endogenous siRNA targets are mis-regulated in *cec-3; cec-6* mutants. Namely, *maternal effect germ-cell defective (meg)* genes involved in regulating germ granule coalescence are up-regulated in *cec-3; cec-6* mutants. Interestingly, genes down-regulated in *meg-3/4* mutants are upregulated in *cec* mutants. While *cec-3; cec-6* mutants have similar germ granule and mortal germline phenotypes to the piRNA Argonaute mutant *prg-1,* our analyses using a germline piRNA sensor suggest that*cec-3* and *cec-6* are not required for piRNA-mediated silencing. We are currently investigating how *cec-3* and *cec-6* impact the small RNA repertoire and transcriptome over generations. Together, our results suggest a role for these heterochromatin reader proteins in the small RNA and chromatin silencing pathways that maintain germ cell fate and genome integrity.

658C **Elucidating the mechanism of mitochondrial DNA copy number decline with age** Arlene Garcia, Maulik R PatelDepartment of Biological Sciences, Vanderbilt University

Introduction

Mitochondria are unique among organelles in that they contain their own genome. The mitochondrial genome is regulated independent of the cell cycle, allowing it to reach levels of hundreds to thousands of copies per cell. We have found that mitochondrial DNA copy number drops precipitously in aging *Caenorhabditis elegans*. Further, we have found that this decline is suspended in developmentally arrested dauer animals. Additionally, it has previously been shown that mitochondrial organelle content increases in aging *C. elegans* due to a decline in mitophagy activity. These results support the hypothesis that the age-related decline in mtDNA copy number is actively regulated at the sub-organellar level.

Methods and Results

My preliminary results show that mtDNA copy number declines as a function of age in the somatic tissue of *C. elegans*. mtDNA copy number was assessed in the somatic tissue of whole organisms using droplet digital PCR. Sterile, *glp1* mutant animals were used to isolate somatic mtDNA. Similarly, we assessed mtDNA in developmentally arrested dauer animals, which was induced using *daf-2* mutants. We found that the age-related decline in mtDNA copy number does not occur in dauer animals.

Discussion

Given that mtDNA decline is suspended in dauer and that mitochondrial organelle content increases with age, this suggests that the decline in mtDNA copy number cannot be explained by a loss of mitochondrial organelles. To verify that regulation of mtDNA content with age occurs at a sub-organellar level, future studies include normalizing mtDNA copy number to mitochondrial membrane content using fluorescent markers. If mtDNA content is regulated at a sub-organelle level, potential mechanisms explaining the age-related decline include nuclease enzymes, such as the exonuclease domain of polg-1 and cps-6, and the transcription factor and packaging protein *hmg-5*. Future steps include inhibiting these processes and assessing their impact on mtDNA levels.

659C **ATPase Function of SMC proteins in Chromosome-wide Gene Regulation** Bahaar Chawla[1], Suchi Jatia[2], Dillon Sloan[1], Gyorgyi Csankovszki[11]Molecular, Cellular, and Developmental Biology, University of Michigan, [2]Biochemistry, University of Michigan

Structural Maintenance of Chromosome (SMC) proteins function in condensin complexes, hydrolyzing ATP for energy to condense DNA for cell division. However, their role in interphase gene regulation is not well understood because most mutations in SMC proteins are lethal. *C. elegans* present a unique opportunity to study SMC proteins through their third condensin complex, condensin I^{DC}, which has a unique SMC protein DPY-27, and it works specifically in the dosage compensation complex (DCC) to regulate X-chromosome gene expression. However, the contribution of the ATPase activity by condensin I^{DC} in this chromosomal-wide gene regulation is not known.

We aim to understand this role with *dpy-27* mutant worms that disrupt ATPase function and evaluating the effect on X chromosome structure, gene regulation, and other DCC functions. These studies will help us understand if condensin I^{DC} is a true motor like other condensins or if it only serves as a scaffold for the members of the DCC.

DPY-27, like other eukaryotic SMC proteins, functions in a dimer with another SMC protein, MIX-1. *In vitro* biochemical analysis of DPY-27 and MIX-1 suggest that DPY-27 is a true ATPase. Using *C, elegans*, we generated one mutation in *dpy-27* through CRISPR by mutating the essential glutamate in the Walker B motif to a glutamine, disrupting ATP hydrolysis. Using this mutant, we found that the subunits of condensin I^{DC} localize with each other but result in two populations of condensin I^{DC}; one that is strongly DNA bound and the other is transiently. Further experiments have shown that the X chromosomes in mutant nuclei are significantly decondensed and mutant DPY-27 is not found exclusively on the X chromosomes.

Taken together, our results suggest that losing the ATPase function of condensin I^{DC} causes decreased binding of the DCC to the X chromosome, resulting in improper dosage compensation. Further experiments hope to elucidate the specific mechanisms affected by the ATP hydrolysis mutation and by what degree gene expression of the X chromosomes is altered.

660C **Phosphoproteomics-based identification of an operon involved in oomycete defence in *C. elegans*** Ming Yi[1], Florence Drury[2], Manish Grover[2], Sarai Pacheco[3], Alex Montoya[3], Holger Kramer[3], Enrique Martinez-Perez[3], Michalis Barkoulas[21]Life Sciences, Imperial College London, [2]Imperial College London, [3]MRC London Institute of Medical Sciences

Natural infection of Caenorhabditis elegans by Myzocytiopsis humicola provides an opportunity to study animal host responses to an oomycete infection. We previously characterised the immune response to oomycete detection known as the oomycete recognition response (ORR) and shows that it includes the epidermal induction of *chitinase-like (chil)* genes as its main hallmark. Through forward genetic screens, we have identified an epidermal localized receptor tyrosine kinase named OLD-1 as the key driver of this response. To understand the downstream signaling pathway, we compared using phosphoproteomics WT and *old-1(-)* animals in the presence and absence of oomycete extract, a water-based innocuous preparation from pathogen cultures that is sufficient to activate the ORR. We found 46 proteins to be specifically phosphorylated upon exposure to oomycete extract and 27 of them appeared to be phosphorylated in an OLD-1 dependent manner. Interestingly, two of these proteins namely B0507.8 and B0507.7 are physically located within the same operon on chromosome V and are both induced as a part of the ORR, but also in response to intestinal pathogens such as microsporidia or the Orsay virus. Using CRISPR-genome editing, we deleted this operon and found that animals exhibited significantly reduced induction of *chil* genes upon *old-1* overexpression and were also more susceptible to oomycete infection. This work highlights that these newly identified components may play a role in immunity driving pathogen-specific outcomes in a tissue-restricted way.

661C **An investigation of Argonaute binding specificity and factors that drive germ granule localization within the *C. elegans* germline** Sanjana Rajeev[1], Carolyn M Phillips[21]University of Southern California, [2]Molecular and computational biology, University of Southern California

RNA silencing or RNA interference (RNAi) is a fundamental mechanism by which gene expression is regulated. The RNAi pathway has two main players: small RNAs and Argonaute proteins. Small RNAs are short non-coding RNAs about 18-30 nucleotide in length which are non-functional on their own but when bound to an Argonaute protein, they turn into gene silencing machines by forming the RNA-induced silencing complex (RISC). *C. elegans* are known to have 19 functional Argonaute proteins, many of which are present in the germline and are concentrated in condensates at the nuclear periphery known as germ granules or nuage. At least four distinct compartments are known to exist: P granules, Z granules, *Mutator* foci and SIMR foci each with a specialized function in transcript surveillance and RNA silencing. In this study, we focus on WAGO-1, a secondary siRNA binding Argonaute protein which has been shown previously to localize within the P granule subdomain of nuage (Gu et al., 2009). Additionally, WAGO-1 has been shown to directly interact with ZNFX-1 and MUT-16 which localize to Z granules and *Mutator* foci, respectively (Ishidate et al., 2018; Manage et al., 2020). The mechanism of germ granule localization is unknown. Here we discover that binding to a small RNA is essential for proper localization of WAGO-1 at perinuclear germ granules. We further observe that when WAGO-1's preferred small RNA partners are not available, WAGO-1 displays no ability to bind any other class of *C. elegans* small RNAs, indicating that it has complete specificity for WAGO-class small RNAs. We are expanding this analysis to other germline expressed WAGO Argonaute proteins to learn more about the factors that drive Argonaute protein localization and small RNA binding specificity. This work is ultimately important to understand RNA silencing to regulate gene expression.

662C **Characterizing mRNA polyadenylation sites in *C. elegans*** Emma Murari[1,2], Bridget Diviak[1], Sara Keane[1], Reagan Conrad[1], Marco Mangone[31]School of Life Sciences, Arizona State University, [2]The Biodesign Institute at Arizona State University, [3]The Biodesign Institute, Arizona State University

The cleavage and polyadenylation of pre-mRNAs are critical steps needed for RNA transcription termination and maturation. This process is executed by a large multi-subunit complex known as the RNA cleavage and polyadenylation complex (CPC). The CPC binds to the polyadenylation signal (PAS) element, a conserved hexameric element located at the end of the pre-mRNA's 3' Untranslated Region (3'UTR), scans the downstream sequence (*buffer region*), and then performs the cleavage reaction at the polyadenylation site (PS) element.

Despite their importance, the location and function of most of these elements required for productive RNA cleavage are poorly characterized. Prior research from our lab revealed an enrichment of adenosine nucleotides at the PS site and demonstrated that their removal alters the location of the cleavage site *in vivo*, suggesting an important novel role of the *buffer region* and the PS element in the pre-mRNA cleavage and polyadenylation reaction. Notably, these adenosines are frequently preceded by uracil nucleotides, but their presence and requirement in the cleavage reaction are unclear.

Here we developed several novel *in vivo* and *in vitro* cleavage and polyadenylation assays and studied how the terminal uracil and adenosine nucleotides, the composition and the length of the *buffer region*, and the location of the PS elements influence the location of the RNA cleavage and polyadenylation reaction. Our results validated and expanded our original findings identifying a critical role of the terminal adenosines in the RNA cleavage reaction, suggesting a novel mechanistic role of how this process is executed in higher eukaryotes.

We are now using the results of these assays to produce a working framework to model PS elements and *buffer regions* in the 5,546 genes in the worm transcriptome, which currently lack annotated 3›UTR data (WormBase). Our work will greatly improve our understanding of pre-mRNA cleavage and polyadenylation reaction in *C. elegans* and will allow us to provide a much-needed reference for PS elements in the worm transcriptome to the scientific community.

663C **An updated *C. elegans* nuclear body muscle transcriptome for studies in muscle formation and function** Anna L Schorr, Nicholas A Cuda, Alejandro Mejia, Martina Y Miranda, Marco MangoneArizona State University

The body muscle is an important tissue used in organisms for proper viability and locomotion. Although this tissue is generally well studied and characterized, and many pathways have been elucidated throughout the years, we still lack a comprehensive understanding of its transcriptome and how it controls muscle development and function. Here, we have updated a nuclear FACS sorting-based methodology to isolate and sequence a high-quality muscle transcriptome from Caenorhabditis elegans mixed-stage animals. We have identified 2,848 muscle-specific protein-coding genes, including 78 transcription factors and 206 protein-coding genes containing an RNA binding domain. We studied their interaction network, performed a detailed promoter analysis, and identified novel muscle-specific cis-acting elements. We have also identified 16 high-quality muscle-specific miRNAs, studied their function in vivo using fluorochrome-based analyses, and developed a high-quality C. elegans miRNA interactome incorporating other muscle-specific datasets produced by our lab and others. Our study expands our understanding of how muscle tissue functions in C. elegans and provides results that can be applied to humans to study muscular-related diseases.

664C **Chromodomain Proteins CEC-3 and CEC-6 Restrict Transgenerational Epigenetic Inheritance in a Temperature Sensitive Manner** Phoebe A. W. Bhagoutie, Chengyin Li, Victor Lao, Arneet L. SaltzmanDepartment of Cell and Systems Biology, University of Toronto

Small RNA and chromatin pathways in *C. elegans* are fundamental for the maintenance of proper germline development. Coordination between these pathways protects germline immortality and regulates the transmission of epigenetic information across generations. Notably, the exogenous RNA interference (RNAi) pathway is a useful tool for studying transgenerational epigenetic inheritance (TEI) as RNAi-induced heritable silencing can persist in the absence of the initial RNAi signal. We have previously reported that *C. elegans Chromodomain* (CEC) proteins CEC-3 and CEC-6 promote germline immortality in a temperature-dependent manner. CEC-3 and CEC-6 also recognize repressive histone H3K9 and H3K27 methylation marks indicating their potential role in gene regulation at the chromatin level. However, their specific role in safeguarding the germline has yet to be fully elucidated. Using dsRNA targeting a germline-specific *gfp* reporter in an RNAi inheritance assay, we found that *cec-3;cec-6* mutants display prolonged heritable silencing and increased heterochromatin mark deposition at the target transgene locus. Similar to the temperature-sensitive progressive sterility phenotype in *cec-3;cec-6* mutants, at high temperature (25°C) we also observe an enhanced frequency and duration of heritable transgene silencing. To understand when temperature affects RNAi inheritance, we performed F1 temperature-shift assays. Interestingly, enhanced heritable silencing in *cec-3;cec-6* mutants is dependent on the temperature exposure of the inheriting generations and not the animals exposed to the initial RNAi trigger. Shifting F1 animals to high temperature also reduces silencing inheritance in wild-type but not *cec-3;cec*-6 mutants. The inheritance of silencing in *cec-3;cec*-6 mutants also depends on the activity of the germline nuclear Argonaute *hrde-1* in the inheriting generations. Therefore, our findings suggest that CEC-3 and CEC-6 regulate TEI duration by promoting temperature-dependent resetting of silencing inheritance in a nuclear RNAi-dependent manner to preserve germline integrity.

665C **The germline KH protein, TOFU-7, facilitates post-transcriptional piRNA processing on the mitochondrial surface.** Cole Andrew Pero[1], Redi Metali[1], Craig Mello[2,3]1RNA Therapeutics, University of Massachusetts Chan Medical School, [2]RNA Therapeutics Institute, University of Massachusetts Chan Medical School, [3]Howard Hughes Medical Institute

Proper germline development is essential for the propagation of an animal species. One major, conserved manner in which this

development is secured is though the action of PIWI-interacting RNAs (piRNAs). Although much is known about the production of piRNAs in flies and mammals, less is known about piRNA biogenesis in *C. elegans*, which is distinct at the levels of transcription, post-transcriptional processing, and even the structure of mature piRNAs. Establishing these details will bolster our understanding of the common principles underlying this system and therefore the preservation of germline development. Previous studies have identified several genes involved in piRNA production in *C. elegans*. In particular, the KH-domain protein *tofu-7* was found to be important for the post-transcriptional processing and stability of mature piRNAs. Here we show that *tofu-7* is essential for the stability of the PIWI argonaute PRG-1. Interestingly, TOFU-7 is localized to mitochondrial membranes in the germline, unlike other piRNA-processing factors that have been shown to localize to nuclei or P granules. This localization of TOFU-7 is similar to that of the piRNA processing factor PAPI found in *Drosophila melanogaster* and *Bombyx mori*, which is likewise anchored to mitochondrial membranes and binds to piRNA precursors via its KH domains. PAPI contains a TUDOR domain through which it binds directly to PIWI argonautes. While TOFU-7 lacks this domain and does not appear to directly bind PRG-1, our yeast two-hybrid assays suggest that TOFU-7 interacts with the chaperone HSP-90 which has been implicated in Argonaute loading. Overall, our findings support a role for TOFU-7 in piRNA-mediated silencing and suggest a conserved role for mitochondrial anchoring of piRNA processing and loading.

666C **ZNFX-1 plays a surprisingly complex role in propagating epigenetic states across generations** Daniel Durning[1], Humberto J. Ochoa[1], Takao Ishidate[1,2], Lucas Prescott[1], Craig C Mello[1,21]UMass Medical School, [2]Howard Hughes Medical Institute

The *Caenorhabditis elegans* PIWI Argonaute PRG-1 engages thousands of endogenously encoded piRNAs within perinuclear nuage (P granules). Sufficient base-pairing between piRNAs and their mRNA targets can elicit a silencing response that is maintained transgenerationally by worm-specific Argonautes (WAGOs), even in the absence of continued PRG-1 activity. We previously described ZNFX-1 as a factor involved in the maintenance of piRNA-triggered inherited silencing. However, the biochemical functions of ZNFX-1 in balancing and maintaining inherited silencing remain poorly understood. Here we report that *znfx-1* and *prg-1* work in parallel to promote silencing on both endogenous protein coding genes and on transposons. Additionally, we show that they function together to prevent thousands of endogenous mRNAs from becoming de-novo targets of small RNA templating. Surprisingly, we find that RNAi inheritance, while defective in *znfx-1*, is partially rescued in *prg-1 znfx-1* double mutants. These data suggest that ZNFX-1 promotes but is not essential for the inheritance of silencing. For example, *znfx-1* is required for inherited silencing of *dpy-11* RNAi*,* but inheritance is restored in *prg-1 znfx-1* doubles. Interestingly, *znfx-1* which is only visibly expressed in the germline throughout development is required zygotically (not maternally) for the inheritance of *dpy-11* silencing. Since *dpy-11* is a somatic gene and its function is not provided maternally, this latter finding implies the somatic expression of ZNFX-1 in embryos (not detected by our tagged ZNFX-1 alleles) or suggests some indirect effect of ZNFX-1 activity in the germline on *dpy-11* expression in the hypodermis. We also note that *znfx-1* mutants cause GLH-4 (but not other P granule components analyzed to date) to become de-localized from the P granules. Interestingly, GLH-4 delocalization was also observed in a helicase-dead allele of ZNFX-1 that was itself still localized in P granules. Thus, the helicase function of ZNFX-1 is required to localize GLH-4 (but not its paralog GLH-1) into nuage. Clearly we are just scratching the surface of the intricacy of interactions within nuage that function to maintain epigenetic states across generations.

667C **Investigating an *rde-3*-independent mechanism of piRNA silencing in *C. elegans.*** Wendy Tan, Yuehe Ding, Craig MelloRNA Therapeutics Institute, University of Massachusetts Medical School

Despite the deep conservation of piRNA pathway components and their well-established importance in fertility and genome defense, piRNA pathway silencing strategies diverge across the animal kingdom. In the *Caenorhabditis elegans* germline, the piRNA-associated PIWI homologue PRG-1 recruits the *Mutator* complex to trigger mass production of 22G secondary siRNAs that associate with WAGO Argonautes to silence targets. However, recent comparison of *prg-1* and *Mutator* complex component *rde-3* null germline expression profiles identified several genes that are uniquely upregulated in *prg-1* animals, suggesting that PRG-1 can initiate piRNA silencing via the canonical 22G-dependent pathway and a currently undescribed mechanism that does not require *rde-3(+)* activity. To address *rde-3*-independent silencing, we have generated two independent fluorescent transgene reporters, a piRNA reporter containing several piRNA target sites and a lambdaN-boxB-based PRG-1-tethered reporter. Both piRNA pathway reporters remain silenced in *rde-3* mutants, despite drastic depletion of targeting 22Gs, but became de-silenced in *prg-1, rde-3* double animals. Interestingly, the *Mutator* complex component MUT-16 is required for both 22G-dependent and *rde-3*-independent piRNA silencing pathways, as loss of *mut-16* alone resulted in the full desilencing of the piRNA pathway reporters. Using gonadal mRNA-seq we have identified endogenous genes that appear to be regulated by the *rde-3*-independent piRNA silencing pathway. Future studies aim to validate putative endogenous targets and identify additional components of the *rde-3*-independent piRNA silencing pathway.

668C **The SUMOylation of the chromodomain factor MRG-1 impacts its interaction with chromatin-modifying enzymes** Johan Girgenrath, Craig Mello RTI, Umass Chan Medical School

MRG-1 is a highly conserved chromodomain protein shown to be involved in several processes, ranging from DNA-damage repair, X-chromosome inactivation, and promoting germline fertility. Previously, it has been shown to be involved in the silencing of piRNA reporter transgenes, suggesting its potential role in piRNA-mediated heterochromatin formation. It is thought to bind to chromatin via the activating H3K36me mark, potentially positioning it to dynamically react to piRNA targeting and recruit chromatin modifying enzymes to establish a silent heterochromatin state. In the C. elegans germline, MRG-1 exists both in its native form, as well as a form in which it is modified by the small ubiquitin-like modifier (SUMO). MRG-1's SUMOylation is conserved across organisms, however the role of this modification in MRG-1 function is unknown. SUMOylation has been shown to impact protein-protein interactions, as well as protein stability. We have identified the location of MRG-1 SUMOylation to be the conserved lysine K301. In worms bearing a K301R mutation MRG-1 exists exclusively in an unmodified form. Surprisingly, K301R mutants, unlike null alleles, are viable and fertile, and do not have obvious defects in gene expression or RNA silencing. Preliminary IP studies, suggest that the SUMOylation of MRG-1 may reduce its interaction with members of the NuA4 histone acetyltransferase complex. We are currently searching for additional perhaps subtle phenotypes associated with the K301R lesion that might reveal a role for MRG-1 SUMOylation in regulating gene expression or other events.

669C **Whole genome screening for exopher producing genes from proteostressed neurons** Mark Abbott[1], Ilija Melentijevic[2], Ryan Guasp[2], Monica Driscoll[21]Genetics, Rutgers University, [2]Rutgers University

Protein aggregation is critically implicated in the pathogenesis of most late-onset neurodegenerative disorders. Aggregating proteins disrupt intracellular functions and can spread to proximal neurons, compromising neighboring cells. Elucidating neuroprotective mechanisms that limit proteo-toxicity 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 (~4 µM) 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. The contents expelled in exophers 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. Analogous processes have been identified in murine cardiac and human brain tissue, lending support to exopher production's involvement in human pathology.

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. Exophers are produced relatively infrequently, and without any known markers to distinguish them from other cell components 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 screening a neuronal RNAi-sensitized strain possible. Our analysis of the screen outcome is ongoing but hits to date suggest exophergenesis is integrated with proteasome, autophagy and germline biology to modulate exopher production.

670C **Identifying the regulatory role of intracellular signal transduction in neuronal alternative splicing patterns across development** Michael Zoberman, John Calarco Cell and Systems Biology, University of Toronto

Alternative splicing is a critical regulatory layer involved in modulating gene expression and generating proteomic diversity to establish unique transcriptomic identities in individual tissues and cells. Notably, neuronal tissue contains an abundance of alternative transcripts that contribute to their specialized functions. Intracellular signalling pathways alter cellular function differentially between tissues, developmental stages, and in response to stimuli. There is accumulating evidence that these signalling pathways can exert their effects by regulating alternative splicing patterns. However, the specific cascades and the mechanisms they use to regulate splicing factors as well as their impact on alternative splicing networks largely remain unknown. I am conducting a reverse genetics screen to identify signalling cascades that regulate alternative splicing patterns. The screen will be followed by the identification of signal-regulated splicing factors and the mechanisms used to regulate splicing in a signal-dependent manner.

671C **Characterising novel epigenetic pathway components in *Caenorhabditis elegans*** Carlotta Wills, Alyson Ashe School of Life and Environmental Sciences, The University of Sydney

Epigenetic regulation is becoming of increasing interest in various fields of research, as it is becoming clear that such pathways are involved in a myriad of complex cellular processes that have far-reaching impacts on organisms and populations. Using *Caenorhabditis elegans* as our model of choice, we investigate the molecular mechanisms underpinning epigenetic processes

and seek to characterise the roles various genes play in these pathways.

In the search for readers of H3K23 methylation, a recently identified histone mark of interest, we performed a pulldown using synthesised histone tail peptides as bait and whole *C. elegans* lysate as prey. In subsequent curation of the resulting list of protein hits, we have selected two genes for further investigation, *tag-250* and Y50D4C.3, that we predict are involved in epigenetic regulation. By observing the predicted structures of these genes' protein products as found in the AlphaFold database, we have highlighted previously unannotated structural features of these proteins, which in turn provide novel insights into their potential functions. Relevant structural features include the presence of Tudor domains, which commonly act as readers for histone modifications, as well as a LOTUS domain and an intrinsically disordered region (IDR) in TAG-250 and Y50D4C.3 respectively. Furthermore, Y50D4C.3 shows structural homology to a well-characterised human gene, TDRD3, which has known roles in transcriptional activation. Together, these features suggest that the proteins may act within the realm of epigenetic regulation, likely within complexes and coordination with other proteins.

We aim to investigate and characterise the role of these genes, thereby deepening our understanding of the different roles and interactions of various epigenetic pathways. To this end, we have generated null mutants of these candidate genes to then be used in functional assays, and are working to tag the loci, which will permit further examination of the endogenous gene product. Through investigating how the knockouts of these genes affect *C. elegans* at the epigenomic, transcriptomic and phenotypic levels, and tracking the expression and localisation of the gene *in vivo*, we can obtain an overall picture of the gene's functions within both individual cells as well as the whole organism. We will present our results on this work thus far as well as our future directions in this endeavour.

672C **More than a SET of parts: Probing the establishment of transgenerational epigenetic inheritance by the multi-domain histone lysine methyltransferase, SET-25** Jessica Hawes[1], Natasha Jones[1,2], Dhruv Monteiro[1], Rachel Woodhouse[1,3], Joel Mackay[1], Alyson Ashe[11]University of Sydney, [2]Monash University, [3]Australian National University

Epigenetic signals, such as histone modifications and small RNAs, affect how the genome is read, transcribed, and translated. This extra-genetic information can be conferred across multiple generations through the phenomena of transgenerational epigenetic inheritance (TEI). This is hypothesised to facilitate dynamic adaptation to environmental conditions and the pressures a population faces without inducing permanent genetic change. Utilising a transgenic *gfp*sensor, we have observed robust inheritance of RNAi-induced gene silencing over 6-10 generations. We have used this sensor to characterise genes required for the establishment and maintenance of TEI and isolate requisite stages and mechanisms.

SET-25 is a H3K9 methyltransferase that is necessary for the establishment of TEI in *C. elegans*. Using the sensor assay, we have found that a functional copy of the gene is required only in the exposed animal for normal levels of silencing to be present in offspring, even if these offspring are mutant for *set-25*. Therefore, the contribution of *set-25* to establishing TEI must occur in the P0 animal. H3K9me3 is a silencing mark understood to be deposited by the SET-domain but, despite a decrease in the level of total H3K9me3, inactivation of the catalytic activity of this SET domain does not confer the same extent or severity of TEI deficiency seen in *set-25* null organisms. It is evident that there is something more at play in the involvement of SET-25 in TEI and the search for these structures and functions was the focus of our study.

Using structural prediction tools, such as I-TASSER and AlphaFold®, we identified an ordered region that is similar in sequence to a Tudor domain but has a predicted structure that aligns more closely with a canonical chromodomain. Both Tudor and chromodomains are methyl-lysine and methyl-arginine readers within the 'Royal family' of domains but have distinct functions and interactions with other chromatin-associated proteins. Chromodomains are chromatin reader domains that have also been found, within some organisms, to modulate protein-RNA interactions. Our study of the structure-function relationships within SET-25 breaks the protein down into modules to further probe the mechanism of TEI establishment.

673C **Maintenance of dauer germline quiescence and integrity by AMP Kinase and a putative RNA helicase** Sabih Rashid, Richard Roy Biology, McGill University

Developmental plasticity is a strategy used by many organisms to adapt to environmental stress and improve their chances of survival. The nematode *Caenorhabditis elegans* can adapt to starvation and other stressors by transiting through an alternate developmental stage called the dauer. Dauer larvae are quiescent and able to survive for many months without external nutrition by altering their metabolism. When environmental conditions improve, animals can exit dauer and resume normal reproductive development without issue. However, the genetic mechanisms that ensure animals can retain their reproductive fitness throughout this developmental detour remain unclear. Notably, loss of the AMP-activated protein kinase (AMPK) leads to severely reduced dauer survival, and induces germline hyperplasia and post-dauer sterility, suggesting this major metabolic regulator may be a key factor in ensuring dauer germline quiescence. It is not known, however, how AMPK exerts such control over the germ line in order to ensure animals can survive and remain fertile following periods of nutritional stress.

Through a screen for putative AMPK targets, we identified an uncharacterized RNA binding protein which appears to regulate the dauer germ line in the absence of AMPK. RNA interference or genetic deletion of this protein suppresses the post-dauer sterility of AMPK mutant animals. Interestingly, the protein appears to function in the intestine rather than the germ line to regulate fertility, and analysis of its predicted protein domains suggests it may be a helicase. Deletion of several predicted intrinsically disordered regions (IDRs) within the protein suppresses both its localization and function during the dauer stage, hinting that it may undergo liquid-liquid phase separation. Finally, CLIP-Seq data reveals numerous protein-coding RNAs bind to this target during the dauer stage in an AMPK mutant background. Regulation of these RNAs by the helicase may be responsible for the effects we observe in the germ line during the dauer stage. These findings hint at the complex interplay of genetic signals that must occur to ensure animals are able to effectively transit through periods of environmental challenge, and the role that RNAs and RNA-binding helicases may play in these scenarios.

674C **Characterization of a new isoform of the MEC-8 protein** Camille RONGIER, Denis DUPUYARNA inserm U1212, Université of Bordeaux

In 2020, Tourasse et al. meta analysis of the transcriptome of C.*elegans* and discovered a new isoform of the MEC-8 protein, called MEC-8b, representing 15% of the transcripts. The MEC-8 protein (for abnormal mechanosensation) is encoded by the mec-8 gene carried on chromosome I. mec-8 is required for the proper development of body wall muscles, chemosensory neurons and touch receptors. The canonical isoform of MEC-8 (MEC-8a) has two RNA recognition motifs (RRMs) encoded by exons 1, 2 and 3. 15% of RNA-seq reads, support the existence of the MEC-8b isoform with only one RRM. In which the skipping of exon 3 results in the removal of the stop codon leading to the translation of exon 4.

In order to study the fonction of the MEC-8b isoform, two strains were generated by CRISPR cas-9 expressing either exclusively MEC-8b or MEC-8a. First, a phenotypic characterisation of these mutants was carried out regarding : mechanosensation, embryonic development and oviposition tests.

We characterized the spatio-temporal expression pattern of the 2 isoforms using fluorescent reporters expressed from an extrachromosomal array.

The microscopic analysis results show co-localisation the 2 isoforms from the embryonic to the adult stage.

Key words : alternative splicing – MEC-8 protein

675C **Identifying the factors involved in trans-splicing regulation using SL2 Reporter System** Muhammad Adnan Nawaz[1], Denis Dupuy[21]European Institute of Chemistry and Biology, University of Bordeaux, [2]University of Bordeaux

Spliced leader (SL) trans-splicing is a critical step in mRNA maturation in many eukaryotes, where a short sequence (SL) is transferred from a precursor SL-RNA into the 5’ end of an immature mRNA. This mechanism contributes to the resolution of polycistronic transcripts. Trans-splicing produces hybrid transcripts, which increases the complexity of gene expression and the diversity of the proteome. The majority of research on spliced leader trans-splicing and its relationship to operon gene expression has been undertaken in C. elegans. To uncover the factors involved in trans-splicing, we are implementing a strategy that will rely on the construction of reporter strain in which the expression of a fluorescent protein is conditional to SL2 trans-splicing. This implies that the fluorescent protein will be produced when SL2 trans-splicing happens, allowing us to readily observe and evaluate the trans-splicing activity. This strain will be used for a mutagenesis screen through which we aim to uncover new insights into the mechanisms of trans-splicing regulation. This work will reveal new insights into how operons and gene expression are coordinated in C. elegans and other eukaryotes by investigating the link between SL2 trans-splicing and operon gene expression. Understanding the mechanisms that control trans-splicing may allow us to identify novel therapeutic targets for anti-parasitic treatments.

676C **Investigating Proteins Specific for *C. elegans* snRNA in SL1 *trans*-splicing** Feyisola Fasimoye, Berndt Muller, Jonathan Pettitt, Bernadette Connolly Institute of Medical Science, University of Aberdeen

Spliced leader *trans*-splicing is an essential post-transcriptional event in the gene expression of many important human, animal, and plant parasites. In nematode *Caenorhabditis elegans*, Spliced leader 1 (SL1) *trans*-splicing involves the replacement of the original 5’ end of a pre-mRNA called “outron” by a short RNA sequence (about 22 nucleotide) called “spliced leader donated by a specialised small nuclear non-coding RNA, the SL1 RNA. SL1 *trans*-splicing is a process related to *cis*-splicing and requires a novel RNA-protein particle called spliced leader 1 small nuclear ribonucleoprotein (SL1 snRNP). Previous studies have shown that SL1 RNP is composed of SL1 RNA, SNA-2, SNA-1 and heptameric Sm proteins. SNA-2 is an essential protein with predicted RNA recognition motifs (RRMS) while SNA-1 has no known RNA binding domains. We also include SUT-1, a paralogue of SNA-1, in our investigations.

Here we present our progress in cloning and establishing purification strategies for SNA-1, SNA-2, and SUT-1 proteins. We have used these proteins in GST pulldown assays and find that SNA-1 interacts with SNA-2 protein. We also find that the SNA-2 RRMS are not sufficient for this interaction. We also present progress in characterising these proteins and possible interactions with SL1 RNA.

The overall aim of this research is to provide insights in terms of protein structure, and protein-protein and protein-RNA interactions, with view towards identifying potential new drug targets. The outcome of this work will contribute to a detailed and comprehensive view of a mechanism that is essential to our understanding of a major group of human, animal, and plant parasites.

677C **Drugging the embryo: How eggshell permeabilization helps us investigate the cytoskeletal impact on mRNA movement** Naly Torres[1], Karissa Coleman[2], Erin Osborne Nishimura[21]Cell & Molecular Biology, Colorado State University, [2]Biochemistry & Molecular Biology, Colorado State University

Translation typically occurs in the cytoplasm or at the endoplasmic reticulum. However, the mRNA transcript *erm-1 (ezrin/radixin/moesin)* concentrates at the plasma membrane where the protein it encodes will connect the plasma membrane to the actin cytoskeleton. This molecular event ultimately serves to coordinate cell shape changes. Indeed, multiple other transcripts that encode membrane-associated proteins also concentrate at the plasma membrane, but neither the mechanisms directing their transport nor the reasons for their local translation are well understood. Previously, we determined that *erm-1* mRNA localization to the plasma membrane is translation-dependent and directed by its encoded FERM-domain in its N-terminus. Here, we test whether *erm-1/*ERM-1 localization occurs through active or passive mechanisms, and we explore which cytoskeletal components are required for their transport. To this aim, we developed an eggshell permeabilization strategy, followed by drug treatment and then by a gentle smFISH protocol. This affords us a high-yield method for drug treating *C. elegans* embryos. Using this approach, we tested the effect of Nocodazole (microtubule loss) or Cytochalasin D (actin loss) on mRNA localization. Complementarily, we can further investigate whether motor proteins are involved in mRNA transport process using RNA interference knock downs and smFISH techniques. Our data suggests that dynein, but not kinesin, perturbs mRNA localization though it is still unclear whether this effect is direct or indirect. This work is relevant because impaired mRNA localization in neurons and other cell types causes disease but studying mRNA localization in disease-specific models is challenging. Furthermore, the process of directing translation-dependent localization of mRNA to plasma membranes is a distinct and novel method of performing local translation, one whose mechanisms and impacts we aim to better understand.

678C **Aberrant germline H3K4me3 leads to an increase in *spo-11*- and R-loop-dependent DNA damage** McLean Sherrin, Richard Roy McGill University

In starved *C. elegans*, a transgenerational mortalization of the germ line occurs following extended periods of starvation in the L1 diapause. This is greatly enhanced in mutants that lack all AMPK signalling. Understanding how the loss of this critical protein kinase contributes to this transgenerational epigenetic phenomenon will help us to understand better the mechanistic and genetic dynamics of chromatin changes that occur downstream of starvation.

AMPK blocks the SET-2 methyltransferase from depositing H3K4me3 in the germ line during the diapause. In animals lacking all AMPK signaling, we noted an increase in R-loop abundance; DNA-RNA hybrids that form from nascent RNAs that feed into the transcription bubble. The increase in R-loops is heritable and shares significant overlap with aberrant H3K4me3 marks. These structures can pose a significant threat to genome stability when misregulated. We observe increased R-loops and RAD-51 foci in the primordial germ cells (PGCs) in the L1 larvae and the F2 adult germ line of AMPK mutant animals.

DSBs and RAD-51 foci occur normally in the developing PGCs and the adult germ line. In the adult germ line, SPO-11 is required to make DSBs during normal meiotic progression, leading to a reversible accumulation of RAD-51. We noted that RAD-51 forms large aggregates in the previously starved AMPK mutants which is dependent on the increased abundance of R-loops and SPO-11. Surprisingly, the R-loops themselves are also dependent on SPO-11. In the L1 larvae the RAD-51 is associated with DSBs that are linked to active transcription and the onset of post-embryonic development. However, starved L1 larvae should never have RAD-51 foci in the PGCs, since they are quiescent until the animals begin feeding, but in AMPK mutants we see a large increase in R-loops and RAD-51 accumulation.

We hypothesize that in the absence of AMPK, germline integrity and genomic stability are affected by the aberrant deposition of H3K4me3, which may inadvertently enhance topoisomerase activity along with transcription. R-loops are known to form at DSBs in actively transcribed regions and this may lead to increased DNA damage-dependent cell death. *C. elegans* AMPK mutants reveal that the epigenetic landscape of the germ line must be exquisitely regulated to protect against R-loop-dependent genomic instability.

679C **The transgenerational accumulation of repressive H3K9me2 confers longevity via a D AF-12-dependent and a**

DAF-12-independent pathway Jaime C Croft, Teresa W LeeBiological Sciences, University of Massachusetts Lowell

Recent work has shed light on the importance of chromatin for the transgenerational inheritance of complex traits like fertility and aging. In *C. elegans*, mutations in either of two chromatin modifier genes extends lifespan gradually over many generations: *wdr-5*, which encodes a component of the COMPASS complex, and *jhdm-1*, which encodes a putative H3K9 demethylase. We have previously shown that the transgenerational longevity of both mutants requires the heterochromatin modification H3K9me2, and that H3K9me2 accumulates at genes expressed in the germline. Here, we report on how heritable chromatin changes in the germline may influence aging in somatic tissues. The nuclear hormone receptor DAF-12 is expressed in the adult somatic gonad and promotes lifespan extension via germline-to-soma signaling. We have found that *daf-12* is necessary for the establishment of transgenerational longevity in both mutants: populations of *jhdm-1; daf-12* double mutants or *wdr-5; daf-12* double mutants never become long-lived, even after twenty generations. Therefore, the establishment of longevity requires DAF-12. However, once *jhdm-1* mutants have acquired longevity (after six to ten generations), removing the activity of *daf-12* has no effect on the extended lifespan. In contrast, *daf-12* activity is necessary for *wdr-5* mutant longevity, even after *wdr-5* mutant populations have acquired longevity (after eighteen to twenty generations). These results suggest that, once accumulated, the inheritance of high levels of repressive H3K9me2 can affect lifespan through multiple pathways: one that requires DAF-12, and one that is independent of DAF-12. Future work will investigate whether differences in germline-to-soma communication may also generate differences in organismal health during lifespan extension.

680C **Deciphering the variable relationship between overlapping and distinct foci of TBP-1 and PRDE-1 in piRNA biogenesis** Jackson Roberts, Nancy Sanchez, Valerie Reinke Genetics, Yale University

The Piwi interacting RNA (piRNA) pathway plays a role in maintaining germline integrity and fertility through the suppression of transposons and nonself nucleic acids. More than 10,000 piRNAs arise from a small number of discrete genomic regions on chromosome IV in *C. elegans*. The factors PRDE-1, SNPC-4, TOFU-5, and TOFU-4 are components of the upstream sequence transcription complex (USTC), which binds strongly across the piRNA gene cluster and is found to promote piRNA expression. Components of the USTC complex form distinct overlapping foci in the germ line. Despite the role the USTC complex plays in piRNA biogenesis, the functional relationship between the USTC complex and transcriptional regulators remains poorly understood. Recent literature demonstrated that TATA-Box Protein 1 (TBP-1) physically interacts with PRDE-1, however, the mechanism by which TBP-1 coordinates piRNA expression is unknown. To define the functional relationship between the TBP-1 and PRDE-1 foci, we performed super-resolution confocal imaging in living adult worms. Interestingly, we observed that TBP-1 forms multiple distinct foci in *C. elegans* germline nuclei. Surprisingly, we observe temporal variation in the level of colocalization of TBP-1 and PRDE-1 foci across germline development. Z-stack images at different phases in germline development allowed us to generate 3-D models to observe the extent of colocalization in multiple planes. By performing colocalization analysis, we find that TBP-1 and PRDE-1 foci colocalize in the distal tip and early pachytene stages of germline development, however in mid pachytene we observe no colocalization. We will continue to investigate the source of the observed temporal variation and its significance in piRNA biogenesis. This project will provide insight into piRNA biogenesis and how genomic regulation occurs in a germline-specific manner. This research will also aid our understanding of how existing transcriptional mechanisms have adapted to maintain germline integrity.

681C **mScarlet and split fluorophore mScarlet resources for plasmid-based CRISPR/Cas9 knock-in in *C. elegans*** Gillian V Witten[1], Ella DeMott[1], George Huang[1], Daniel J Dickinson[2], Ryan Doonan[11]Glow Worms, The University of Texas at Austin, [2]Molecular Biosciences, The University of Texas at Austin

Fluorescent tagging allows for the visualization of protein expression *in vivo*. Although CRISPR knock-in of fluorescent tags at endogenous loci has revolutionized the authenticity of a reporter gene, it has its limitations. For example, large fluorophores may disrupt the function of the targeted protein. The recent development of split fluorophores could be a great alternative for low molecular weight proteins that are significantly smaller than the size of a full-length fluorescent protein tag. The two fragments of split fluorophores fluoresce only when they assemble *in vivo*. The smaller fragment serves as a “tiny tag” for the protein of interest, whereas the larger fragment is expressed separately. Currently, the best available red fluorescent protein for the visualization of expression *in vivo* is mScarlet. To determine the visibility and authenticity of expression with the use of a tiny tag, we cloned versions of mScarlet and split fluorophore wrmScarlet into the SEC-based system of plasmids for CRISPR/Cas9 knock-in. We then used these tools to tag 4 proteins of high interest that are either considered small or are not completely functional when tagged with a full-length fluorophore: EGL-1, HIS-72, MTL-1, and PTL-1. While our current findings suggest that target protein function is uninterrupted by the split fluorophore, most of these proteins were not able to be visualized. Thus, split fluorophore tags possess many limitations in their use as endogenous reporters.

682C **Characterization of *nhr-25* DNA Binding Domain mutant with CUT&Tag** Belle Ange C Itetere[1], Kimberley T Muchenje[2], Deborah M Thurtle-Schmidt[31]Davidson College, [2]California Institute of Technology, [3]Biology, Davidson College

Mutations in TFs, 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 optimizing CUT&Tag for *C. elegans*. Preliminary results on wild-type, endogenously-tagged NHR-25 resulted in successful recovery of chromatin from L1 and L3 worms, allowing rapid whole-genome profiling of transcription factors from low numbers of C. elegans. We are now poised to endogenously tag the *nhr-25(ku217)* allele to perform CUT&Tag on the mutant allele. By profiling the binding of a mutated transcription factor we can determine how a single nucleotide polymorphism alters TF function.

683C **Genome-wide mapping and analysis of the transposon landscape of *C. elegans* isolates** Cora Albers, Zachary D. Bush, Diana E. LibudaUniversity of Oregon

Transposable elements (TEs) are mobile DNA sequences that can replicate and propagate themselves independently of the host genome. In Caenorhabditis elegans, TEs comprise a significant portion of the genome and their activity is highly regulated to prevent mutations and chromosomal rearrangements. Although TEs play an important role in genetic variation and the evolution of genome structure and function, holistic computational tracking and identification of TEs within genomes is challenging. Previously developed techniques to map TEs focused on individual classes and families, but recent technological advances have enabled the annotation of all known TE families, and provide comprehensive and detailed information regarding the genome-wide distribution and landscape of specific TE families. Using our recently completed de novo genome assemblies for two genetically divergent Bristol and Hawaiian strains of C. elegans and new computational TE annotation tools, we mapped and characterized the TE content of these two newly assembled genomes. Our computational analysis identified over 18,000 TEs in both the Bristol and Hawaiian genomes. Each C. elegans isolate displayed differences in the global distribution of TEs throughout the genome and in the overall amount of each specific TE class within each genome. These results suggest that diversity of TE positioning can contribute to intra-species variation in genomic structure. Further, we identified and mapped transposon superfamilies, such as Zator, that have been relatively under characterized in C. elegans. We found that the Zator transposon superfamily constitutes the majority of TEs identified in both genomes, and that the Bristol genome has 20.6% more TEs from the Tc1/mariner family than the Hawaiian genome. Using specific TEs with unique single nucleotide polymorphisms, we also tracked the movement of individual TEs between the Bristol and Hawaiian genomes. While we found that most TEs did not move between the two lineages, we did identify 38 TEs that moved intrachromosomally and 9 TEs that moved interchromosomally. These results suggest a conservation of TE regulation since these isolates are roughly separated by 50,000 generations. These robust annotations and tracking of TEs between worm isolates illustrate how transposition could play an important role in intra-species diversity and provide insights into the importance of TE regulation on genomic structural variation and gene regulation.

684C **Germline silencing of the X chromosome revealed by CRISPR knock-in in *C. elegans*** Stephen Pullman[1], Chandi Mulchand[1], Daniel J Dickinson[2], Ryan Doonan[11]Glow Worms, The University of Texas at Austin, [2]Molecular Biosciences, The University of Texas at Austin

Self-excising-cassette (SEC)-based CRISPR knock-in is a powerful and scalable system for creating endogenous fluorescent protein tags in *C. elegans*. When tagging several genes using this approach, we found that the Cre recombinase encoded by the SEC sometimes appears to not be expressed following heat shock. As a result, the SEC fails to self-excise, preventing creation of the endogenous tag from the CRISPR insertion intermediate. This happened for the following 6 genes: *asp-3*, *asp-4*, *F13E6.1*, *hum-6*, *pek-1*, and *sod-3*. Interestingly, all of these genes are located on the X chromosome, suggesting X chromosome transgenes are silenced (specifically Cre recombinase in the SEC). To confirm this, we used a strain of *C. elegans* with a secondary autosomal-tagged gene to express Cre recombinase via its SEC, allowing for co-excision of the SEC from the X chromosome gene. Because this approach is very tedious, we have been looking for other solutions to this problem. One possibility is to bypass the germline silencing by using a stable array that contains a non-suppressable version of Cre [i.e. Cre (PATCs), pMDJ22]. We have created a transgenic line of *C. elegans* that expresses Cre (PATCs) from a stable extrachromosomal array. We hypothesize that the Cre expressed from the array will be able to reliably excise the SEC from the X chromosome, resulting in often elusive endogenous tags on the X chromosome.

685C **miRNA Expression and Strand Selection Throughout *C. elegans* Development** Dalton Meadows[1,2,3], Anna Schorr[1,2,3], Hailee Hargis[3,4], Jillian Murray[3,4], Marco Mangone[1,3,51]School of Life Sciences, Arizona State University, [2]Molecular and Cellular Biology Graduate Program, [3]The Biodesign Institute, [4]School of Molecular Sciences, Arizona State University, [5]Biology Graduate Program

MicroRNAs (miRNAs) are 17-22 nucleotide non-coding RNAs that regulate gene expression by targeting non-complementary elements in the 3' untranslated regions (3'UTRs) of mRNAs. miRNAs, which form complex networks of interaction that differ by tissue and developmental stage, display conservation in their function across metazoan species. Yet much remains unknown regarding their biogenesis, localization, strand selection, and their absolute abundance due to the difficulty of detecting and amplifying such small molecules.

Here, we used an updated HT qPCR-based methodology to follow miRNA expression of 5p and 3p strands for all 190 *C. elegans* miRNAs described in miRBase throughout all six developmental stages in triplicates (total of 7,410 experiments), and studied their expression levels, tissue localization, and the rules underlying miRNA strand selection. Our study validated previous findings and identified novel, conserved patterns of miRNA strand expression throughout *C. elegans* development, which at times correlate with previously observed developmental phenotypes. Additionally, our results highlighted novel structural principles underlying strand selection, which can be applied to higher metazoans.

Though optimized for use in *C. elegans*, this method can be easily adapted to other eukaryotic systems, allowing for more scalable quantitative investigation of miRNA biology and/or miRNA diagnostics.

686C **Mapping regulatory elements across nematode taxa to detect reprogramming** Thomas D King, Michael S Werner
School of Biological Sciences, University of Utah

Enhancers and promoters appear to play different roles: promoters strongly activate transcription near the beginnings of genes, and enhancers impart spatial and temporal specificity to gene expression. However, both regulatory elements share important structural features, and both elements induce bidirectional transcription in many taxa, including nematodes. These similarities suggest that enhancers and promoters share a common evolutionary origin -- and that promoters could be reprogrammed into enhancers, or vice versa, over evolutionary time.

Our goal is to identify instances of these transitions, and identify and test the mechanisms by which they occur. We are mapping enhancers and promoters in several nematode taxa: *C. elegans* strain N2, *C. elegans* strain CB4856, *C. briggsae*, *C. tropicalis*, and *P. pacificus*. I have conducted internally calibrated ChIP (Ice-ChIP) to quantitatively map histone modifications characteristic of enhancers and promoters (H3K4me1, H3K4me3, and H3K27ac) in these taxa. I am also conducting capped small RNA sequencing in the same taxa to locate enhancers and promoters based on their characteristic bidirectional transcription patterns. Together, these datasets will provide a high-confidence annotation of enhancers and promoters across nematode taxa representing several different degrees of evolutionary divergence. I will identify examples where syntenic regulatory elements appear to have been reprogrammed, and look for correlations between these events and changes in histone modifications, DNA sequence changes, changes in expression patterns, and birth and death of nearby genes. I hope to gain insight into the mechanisms of regulatory element reprogramming, and I also hope to offer my regulatory element annotations as a resource for other researchers interested in the control and evolution of gene expression.

687C **High throughput identification of Genetic Modifiers: A bioinformatics approach with Machine Learning** K. M. Tahsin Hassan Rahit[1,2], Tatiana Maroilley[1,2], Afiya Chida[1,2], Filip Cotra[1,2], Victoria RA Barbosa[1,2], Maja Tarailo-Graovac[1,21]Biochemistry & Molecular Biology, University Of Calgary, [2]Alberta Children's Hospital Research Institute (ACHRI)

Genetic modifiers are the variants that can modulate another variant's phenotype. In model organisms such as *C. elegans*, the forward genetic screen using genome-wide mutagenesis is a widely used approach to search for modifiers (i.e. suppressors). However, the identification of modifier variants has relied on time-consuming backcrossing strategies. Previously, we showed that short-read Whole Genome Sequencing (srWGS) sequencing is a viable genome-first approach for identifying intragenic modifiers, allowing high-throughput modifier screening in a time and cost-effective manner. When the genome-first approach is applied to find both intragenic and extragenic modifiers, the processing and analyses of srWGS data requires multi-disciplinary expertise, computational resources and time to develop dedicated bioinformatic workflows that could bottleneck a wider use of srWGS for modifier variant detection.

This limitation motivated us to develop a pipeline and a machine-learning (ML) tool for identifying genetic modifiers.

srWGS data analysis consists of multiple steps that broadly include quality control, reference genome alignment, and variant calling and annotation. Each step involves different bioinformatics tools, and their parameters must be tailored. To make srWGS

data analysis accessible to others, we developed a Galaxy-based pipeline called Model Organism Modifier (MOM) that features a user-friendly graphical interface. With MOM, users provide the raw genome reads (fastq file), and the pipeline produces a list of candidate variants with useful annotations for modifier identification.

Furthermore, to avoid manual curation for prioritizing modifiers, we have developed a ML model named ModSpy to prioritize modifier variants. We trained and tested the model using *zyg-1* suppressor srWGS data of experimentally confirmed suppressors. The ML model was validated using srWGS data beyond *zyg-1*. In all cases, ModSpy model has reduced the candidate modifier lists by 90%.

To summarize, a comprehensive understanding of modifiers could explain much of the non-linearity between the genotype-phenotype relationship. MOM workflow combinedly with the novel ModSpy model would substantially reduce the burden of technicality compared to the traditional backcrossing method. Therefore, our advanced bioinformatics-based methods would allow more efficient usage of the genome-first approach for high-throughput identification of the modifiers.

688C **The 959 nematode genomes initiative** Martha Mulongo, Lewis Stevens, Manuela R Kieninger, Pablo Gonzalez de la Rosa, Erna King, Mark BlaxterTree of Life, Wellcome Sanger Institute

Despite considerable advances in sequencing technologies in recent times, only a handful of nematode species are represented by high-contiguity genome assemblies showing chromosomal resolution.

Our project aims to generate high quality reference genomes of nematode species using current state of the art sequencing and assembly technologies. By combining long-read HiFi and HiC sequencing data we are able to provide chromosome-scale reference genomes for all ~300 nematode species currently in laboratory culture, but also for nematode species we sample on field trips across the United Kingdom.

For culturable nematode species, we use inbreeding and bulk sequencing for generating sequencing data. For non-culturable nematodes like marine or parasitic species, we have successfully established a single nematode input protocol which is successful for nematodes as small as *C. elegans.*

Our new assemblies show a substantial increase in contiguity and biological completeness when compared to short-read data assemblies. For example, our new reference genome for *C. afra* has a scaffold N50 of 10.2 Mb, compared to 62 kb for the short-read assembly, and our *Diploscapter coronatus* genome has a scaffold N50 of 87.7 Mb, compared to 1 Mb for the previous assembly.

The generation of such high-quality genome data allows us to study chromosome evolution in the phylum nematoda and detect previously unknown genome regulation events like programmed DNA elimination.

689V **Traditional balancers in *C. elegans*: multi-omics to uncover precise genomic mapping, structure and effect on gene expression** Tatiana Maroilley[1,2], Stephane Flibotte[3], Francesca Jean[1,2], Victoria Rodrigues Alves Barbosa[1,2], Andrew Galbraith[1,2], Afiya R Chida[1,2], Filip Cotra[1,2], Xiao Li[1,2], Larisa Oncea[1,2], Mark Edgley[4], Don Moerman[4], Maja Tarailo-Graovac[1,21]Departments of Biochemistry and Molecular Biology and Medical Genetics, Cumming School of Medicine, University of Calgary, [2]Alberta Children's Hospital Research Institute, University of Calgary, [3]UBC/LSI Bioinformatics Facility, University of British Columbia, [4]Department of Zoology, University of British Columbia

Genetic balancers are complex variants that allow lethal or sterile mutations to be stably maintained in a heterozygous state by suppressing crossover events. They constitute an invaluable tool in the *Caenorhabditis elegans* (*C. elegans*) scientific community and have been widely used for decades. The first/traditional balancers were created by applying X-rays, UV, or gamma radiation on *C. elegans* strains, generating random genomic rearrangements. Their structures were explored with low-resolution genetic techniques (e.g., fluorescence *in situ* hybridization or PCR) before genomic mapping and molecular characterization through sequencing became feasible. As a result, the precise genomic location and nature of most chromosomal rearrangements remained unknown. Using short-read whole-genome sequencing (srWGS) and tailored bioinformatic analyses, we mapped at base-pair resolution the breakpoints and interpreted the structure of 21 chromosomal balancers. We experimentally validated their breakpoints using PCR and Sanger sequencing. Many of the balancers were found to be more intricate than previously described. For instance, we uncovered that *nT1* previously described as a stable reciprocal translocation (IV;V) in fact involved 13 breakpoints mapped on chromosomes IV and V with a structure similar to a chromoplexy. We also showed that *sC4* known to balance the right end of chromosome V was a 3 Mb deletion of the right end of chromosome V and a chromosome fusion (IV;V). Furthermore, srWGS revealed additional structural variants (SVs) and complex genomic rearrangements not known to be part of the balancer genomes allowing us to create the first catalog of large variants in *C. elegans* (>100 variants) including inversions, deletions, duplications, and chromoanagenesis. Next, to better understand the biological effect of balancers and additional SVs,

we used RNA-Seq to explore the impact of breakpoints on gene expression and splicing for a subset of strains. Altogether, our study provides a comprehensive resource of complex genomic variations in *C. elegans,* with a unique focus on the impact of such disruption on transcription and gene expression. It also highlights the power of srWGS to study the complexity of genomes by applying tailored analyses.

690V **The nuclear Argonaute HRDE-1 directs target gene re-localization and shuttles to nuage to promote small RNA mediated inherited silencing** yuehe ding, Humberto Ochoa, Takao Ishidate, Craig MelloRTI, UMass Chan Medical School

Argonaute small-RNA pathways engage heterochromatin-silencing co-factors to promote transgenerational inheritance in animals. However, little is known about how heterochromatin and small-RNA pathways interact to transmit silencing. Here we show that the induction of heterochromatin silencing in C. elegans by RNAi or by artificially tethering pathway components to target RNA correlates with the co-localization of the target alleles in pachytene nuclei. Tethering the nuclear Argonaute WAGO-9/HRDE-1 induces heterochromatin formation, but also functions independently to induce small-RNA amplification. We show that HRDE-1 shuttles to nuage domains called mutator foci where amplification is thought to occur. Tethering a heterochromatin-silencing factor, NRDE-2, induces heterochromatin silencing and also induces the de-novo synthesis of HRDE-1 guide RNAs, and through HRDE-1 acts to further amplify downstream small-RNA silencing. Our findings support a model in which HRDE-1 functions both upstream, to initiate heterochromatin silencing, and downstream, to stimulate small-RNA amplification, establishing a self-enforcing mechanism that propagates silencing to offspring.

691V **Transgenerational silencing of paternal *sid-1* expression is suppressed by maternal *sid-1* expression** Andrei Shubin, Craig P Hunter Molecular and Cellular Biology, Harvard University

Post-fertilization transcriptional activation in *C. elegans* occurs during the maternal-to-zygotic transition at the 4-cell stage. This also corresponds to the beginning of maternal mRNA decay. The link between maternal mRNA and zygotic genome activation is not understood.

Our studies identify maternal *sid-1* mRNA as a factor necessary for the transcriptional activation of the paternally provided *sid-1* allele. In the absence of maternal *sid-1 mRNA*, a wild-type paternal *sid-1* allele is silenced and remains silenced for four subsequent generations. This silencing is not observed in progeny of *prg-1*-mothers. The initiation and maintenance of silencing do not require chromatin-level interaction between alleles but rather depend on the absence of maternal transcript or agents derived from it in the oocyte cytoplasm.

Interestingly, unlike the silencing induced by the introduction of the extrachromosomal *sid-1* promoter transgene array[1], which affects the entire locus (*sid-1* and two upstream genes), the absence of maternal *sid-1* transcript only silences the paternal *sid-1* gene but not the entire paternal locus.

These findings suggest a crucial role of maternal mRNA transcripts in the transcriptional activation or licensing of paternal genes, with PRG-1-associated piRNAs as the likely suppressing agents. Additionally, our results imply a regulatory interaction between transgenerational transcriptional suppression mechanisms that protect against the expression of potentially harmful paternally derived alien genes or selfish genetic elements such as transposons and licensing of paternal genes by maternal mRNA.

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692V ***puf-9* promotes clearance of miR-35-41 microRNAs and somatic sex determination in *C. elegans*** Amelia F Alessi[1], Danny Yang[2], Mallory A Freeberg[3], Margaret R Starostik[1], John Kim[11]Biology, Johns Hopkins, [2]University of Michigan, [3]Johns Hopkins

The miR-35-42 family (miR-35Fam) of microRNAs is required for somatic sex determination (SSD), embryonic viability, and fertility in *C. elegans.* miR-35Fam is highly expressed in the early embryo, where it regulates SSD timing by silencing the SSD pathway genes *sup-26* and *nhl-2* (McJunkin 2014 *G3;* McJunkin 2017 *G&D).* Later in embryogenesis and beyond, miR-35Fam is rapidly cleared by an incompletely understood mechanism. To identify factors that regulate miR-35Fam clearance, we performed a targeted RNAi screen of genes encoding exonucleases and RNA binding proteins (RBPs) and assessed miR-35 levels in L1d larvae. We identified *puf-9* as a potential positive regulator of miR-35Fam clearance. *puf-9* mutants have ~10-fold more miR-35 in L1d than wildtype animals. In addition, loss of *puf-9* significantly enhances the severity of somatic masculinization in *her-1(n695)* gain-of-function animals, indicating that wild-type *puf-9* functions to promote SSD. *her-1* mRNA is also ~2.5 fold upregulated in *puf-9(-)* in conjunction with ~8 fold average upregulation of the *mir-35-41*-dependent pseudomale genes. PUF-9 is part of the con-

served Pumilio/FBF family of RBPs that regulates mRNAs by translational repression and/or mRNA degradation. To determine how *puf-9* promotes miR-35Fam clearance and SSD, we identified mRNAs bound by PUF-9 in embryo and L1d by HITS-CLIP. Like other Pumilio proteins, PUF-9 preferentially binds the 3'UTR of mRNAs enriched in UGUA-containing motifs, including *sup-26* and *nhl-2*. Thus far, we have established that *sup-26* and *nhl-2* are both required for *puf-9*-dependent miR-35 clearance and SSD genetic and molecular defects. Interestingly, *puf-9(-)* exhibits only modest upregulation of *sup-26* or *nhl-2* mRNA levels, suggesting that PUF-9 may regulate *sup-26* and/or *nhl-2* via translational repression. We are currently investigating if SUP-26 and NHL-2 protein levels are altered in *puf-9(-)* and whether PUF-9 directly regulates *sup-26* and *nhl-2* via binding sites identified by HITS-CLIP. Taken together, we propose a model whereby PUF-9 and the miR-35Fam co-regulate *sup-26* and *nhl-2* to ensure robust timing of an essential embryonic developmental program and that *puf-9* promotes miR-35Fam clearance through a target-dependent feedback mechanism.

693V **The role of stress responder ATF-4 in regulating innate immunity in C. elegans** Shawndra Wibisono, Phillip Wibisono, Jingru SunTranslational Medicine and Physiology, Washington State University

Loss of the stress-response transcription factor ATF-4 compromises innate immunity in Drosophila melanogaster exposed to various bacterial pathogens (Vasudevan et al. 2017). However, we found that Caenorhabditis elegans carrying an *atf-4(ok576*) mutation live significantly longer than wild-type animals in response to *Salmonella enterica* SL1344 and *Serratia marcescens* Db11. This long-lived phenotype is absent when *atf-4(ok576)* mutants are exposed to *Pseudomonas aeruginosa* PA14, *Enterococcus faecalis* OG1RF, *Staphylococcus aureus* MSSA 476, or *Streptococcus pneumoniae* SP003. To gain insights into the role of ATF-4 in defense against pathogens in C. elegans, we selected S. enterica SL1344 as an infecting agent. We observed no significant differences in pumping rates, defecation rates, colony forming units, and lawn avoidance behavior between wild-type animals and *atf-4(ok576)* mutant animals. The lifespan of *atf-4(ok576)* mutants is comparable to that of wild-type animals when fed heat-killed *S. enterica* SL1344, suggesting live bacteria are required to induce innate immune response in C. elegans. Several evolutionarily conserved innate immune signaling pathways and stress response pathways have been identified in C. elegans, including the p38/PMK-1 mitogen-activated protein kinase (MAPK) pathway, the DAF-2/insulin-like receptor pathway, the DBL-1/transforming growth factor β (TGF-β) pathway, the unfolded protein response (UPR) pathway, and the integrated stress response (ISR) pathway. We are in the process of investigating how stress responder ATF-4 regulates these pathways to mediate innate immunity against pathogen infections. Our study is likely to identify novel molecular mechanisms underlying host-pathogen interactions.

694V **Characterizing *C. elegans* male germline-mediated transgenerational learned pathogen avoidance and behavior** Katherine S Morillo[1], Rachel Kaletsky[1], Coleen Murphy[21]Princeton University, [2]Molecular Biology, Princeton University

Biological sex regulates an organism's internal and external state. The nematode *C. elegans* has two natural sexes, XX hermaphrodites and XO males. These two sexes exhibit differences in neuronal gene expression and neuroconnectivity, resulting in differences in behavior, such as exhibiting divergent responses to external cues.[1] We previously found that *C. elegans* hermaphrodites exposed to pathogenic *Pseudomonas aeruginosa* (PA14) learn to avoid this toxic bacterium and can pass learned avoidance to several generations of progeny through a small RNA-mediated mechanism that requires an intact germline and neuronal signaling.[2,3] Additionally, PA14 avoidance can be transferred horizontally from exposed animals to naïve animals through virus-like particles encoded by the *Cer1* retrotransposon.[4] This discovery suggests that *C. elegans* has co-opted a potentially deleterious retrotransposon to protect itself and its progeny from infectious agents. Interestingly, the transmission of information across generations through non-genetic means, or transgenerational epigenetic inheritance (TEI), was long thought to be impossible due to the Weismann barrier between the germline and somatic cells, which preserves immortal germ cells in their pristine state. However, recent studies in worms, flies, and mice show that the inheritance of stress responses can help animals survive in harsh environments.[5] Yet, much is left unexplored, such as the role of sex in transmission and inheritance of these stress responses. Here, we will determine: (1) how many generations pathogen avoidance persist in hermaphrodites when inherited from a male parent, (2) if the same neuronal networks mediate inheritance and behavior in male progeny, and (3) whether males are able to learn and inherit avoidance behavior. This work aims to characterize transmission of TEI by the *C. elegans* male germline and define male avoidance behavior to ultimately determine how biological sex mediates TEI of pathogenic avoidance.

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695V **Mathematical Modeling and Computer Simulation of microRNA Regulation Reveals Consequences of Target Site Evolution on Developmental Timing in Roundworm Nematodes** Catherine Campbell, Ramon HerreraBaylor School

One of the hallmarks of life is an organism's ability to grow and develop on schedule. In the roundworm nematode, *Caenorhabditis elegans*, postembryonic development occurs as a progression of four stages that are punctuated by molts. The timing of these molts is governed by a system of highly conserved regulatory genes that control developmental transitions through discrete molecular interactions. This network of genes controls the molt from the first to the second larval stages (L1 , L2), which involves the *lin-4* microRNA (miRNA) targeting the *lin-14* messenger RNA (mRNA) for down regulation. Regulation of *lin-14* occurs through the *lin-4* miRNA binding to two different types of sites in the *lin-14* mRNA 3'UTR. If *lin-4* is bound to a less complementary site, it forms a bulge, and the interaction promotes mRNA degradation. Alternatively, if *lin-4* binds to a site with more complementarity, it prevents LIN-14 protein production through translational silencing of the mRNA. However, it is not known how the both types of binding sites together contribute to heterochronic development and evolution. To explore this question, we built a computer simulation that recapitulates *lin-14* repression by the *lin-4* miRNA. We built a rule-based mathematical model based on the genetic interactions of the molecular species involved in the *lin-4* and *lin-14* interaction using parameters found in scientific literature. Through experimental models, we determined that not only is the absolute number of sites important, but also the proportion of bulged to non-bulged sites that influence the timing of developmental transitions. This suggests that distinct consequences in heterochronic development result from miRNA target site evolution. A comparison of the model outputs based on the predicted number of bulged and non-bulged sites for four other *Caenorhabditis* with the *C. elegans* suggests *lin-4* target site evolution drives changes in developmental timing in the first larval stage nematodes.

696V **Histone Demethylase AMX-1 Regulates Fertility in a p53/CEP-1 Dependent Manner** Xiaojing Ren[1], Hyun-Min Kim-[21]Tianjin University, [2]DNAS, Duke Kunshan University

Histone methylation shapes the epigenetic configuration and adjusts fundamental nuclear processes, including transcription, cell cycle control and DNA repair. The absence of histone demethylase *C. elegans* SPR-5 leads to progressive fertility defects and reduced brood size. Similarly, *C. elegans* LSD2 homolog AMX-1 has been implicated in regulating H3K4me2 and maintaining interstrand crosslinks susceptibility.

This study investigated the histone demethylase AMX-1 in *C. elegans* and uncovered how *amx-1* contributes to sterility in a p53/CEP-1-dependent manner. While sterility in *spr-5* mutants exhibited progress over generations, *amx-1* mutants displayed non-transgenerational fertility defects. Also, *amx-1* mutants exhibited reduced sperms and produced low brood size or sterile worms that retain neither sperms nor germline nuclei, suggesting that fertility defects originated from germline development failure. Surprisingly, sterility exhibited in *amx-1* was mediated by p53/CEP-1 function. Consistent with this result, upregulation of Piwi expression in *amx-1* mutants suggested that AMX-1 is essential for germline development by regulating Piwi gene expressions. We propose that AMX-1 is required for proper Piwi expression and transposon silencing in a p53/CEP-1 dependent manner; thus, the absence of AMX-1 expression leads to defective meiotic development and sterility. This study elucidates how LSD2/AMX-1 contributes to sterility, expanding the boundaries of histone demethylase function.

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697V **Neuronal GPCR NMUR-1 regulates immune response by promoting the expression of energy production proteins in *Caenorhabditis elegans*** Phillip Wibisono, Dodge Baluya, David Gang, Jingru Sun Washington State University

The neuronal G protein-coupled receptor NMUR-1, a homolog to the mammalian neuromedin U receptor, has been implicated in the specificity of *Caenorhabditis elegans* innate immune response against pathogen infections. NMUR-1 controls *C. elegans* transcription activity by regulating transcription factors, which, in turn control the expression of distinct defense genes. This study further investigates the role of NMUR-1 at the protein level in regulating the innate immune responses against *Salmonella enterica* and *Enterococcus faecalis* by utilizing mass spectrometry-based quantitative proteomics. We found that NMUR-1 regulates a class of proteins responsible for transmembrane transport during infection. Specifically, a group of proteins forming F_1F_0 ATP synthase responsible for ATP biosynthesis is downregulated in NMUR-1 loss of function mutants during both *S. enterica* and *E. faecalis* infections. Functional assays demonstrated that inhibited F_1F_0 ATP synthase using RNA interference or chemical modification increases the survival of wild-type *C. elegans* during *S. enterica* infection and *nmur-1* mutants during *E. faecalis* infection. ATP measurements further uncovered that *nmur-1* mutants have a reduced ability to regulate ATP production in response to infections. Overall, our study reveals that NMUR-1 regulates energy production at the protein level against specific pathogens as part of the innate immune response.

698V **Investigating the Localization and Functions of Intestinal Secondary Argonautes** Madeline Prevec, Robert X Lao,

Ruxandra Molnar, Aida Sobhani, Samantha Ormesher, Uri Seroussi, Julie M ClaycombMolecular Genetics, University of Toronto

The nematode *Caenorhabditis elegans* uses small RNA (sRNA) and Argonaute proteins (AGOs) to regulate the expression of target transcripts in a process called RNA interference (RNAi). Remarkably, the worm is capable of taking up double-stranded RNA (dsRNA) from the environment via the intestine and transporting them to distant tissues to elicit RNAi systemically, in processes called environmental and systemic RNAi, respectively. Three AGOs in *C. elegans* localize to the apical membrane of the intestine—PPW-1, SAGO-1, and SAGO-2 (the intestinal Secondary AGOs, iSAGOs). Their apical intestinal localization places them at an interface with the environment, which could allow them to be involved in the uptake of dsRNA and sRNA from the environment, and the transmission of RNAi signals to other tissues in the worm. My project aims to identify how the iSAGOs localize to the apical membrane of the intestine. I will determine the endogenous pathways and environmental factors (e.g. food source, pathogens, etc.) required for localization of iSAGOs to the apical membrane and will disentangle how this localization pattern relates to the endogenous function(s) of iSAGOs in sRNA pathways. Importantly, loss of the iSAGOs leads to RNAi deficiency (Yigit *et al*., *Cell*, 2006). Therefore, we also aim to understand how the iSAGOs could be involved in transmitting RNAi signals between tissues and are using systemic RNAi assays to uncover these roles. Altogether, these studies will shine a light on how AGO/small RNA pathways interpret environmental cues and communicate this information to the rest of the worm.

699V **Elucidation of the link between RNA maturation and neurodegeneration by a cell-specific transcriptomic analysis in *C. elegans*** SARA SAVAHELI, Denis Dupuy University of Bordeaux

Spinal muscular atrophy (SMA) is a neuromuscular disease that causes the specific loss of lower motor neurons (MNs) in affected patients. Mutations in the SMN-1 gene (Survival of Motor Neuron 1) cause ~95% of all SMA cases. SMN-1 controls the assembly of small nuclear ribonucleoproteins (snRNPs) essential for pre-mRNA splicing. SMN protein is ubiquitously expressed in the body and has a variety of roles in addition to its snRNP role: RNA metabolism and transport, DNA repair, and recombination… . It is not clear why MNs are especially sensitive to SMN depletion. We are exploring the molecular origins of the distinct sensitivity of MNs to loss of SMN1 in a *C.elegans* model using a neuron-specific RNAi to selectively knock-down smn-1 in 19 GABAergic VD/DD motor neurons or in touch receptor neurons (TRNs) composed of PLM L/R, ALM L/R, PVM, AVM. We used reporter strains that express fluorescent reporters in the desired cells and crossed them with strains expressing *smn-1* RNAi in the MNs or TRNs. Targeted neurons will be isolated to generate cell specific cDNA libraries for transcriptome sequencing. We will perform a comparative analysis of neuronal transcriptomes following *smn-1* depletion. We will also, identify differential protein interactions involved in neuron survival using TurboID enzyme that can add biotin to proteins that come in close proximity. We expressed SMN1 fused to TurboID in MNs and TRNs. Biotin-tagged proteins will be purified using streptavidin-beads, and will be identified by mass-spectrometry.

Preliminary imaging results indicate that DDs and PLM/ALM do not display the same phenotype as VDs and PVM/AVM when they are submitted to targeted *smn-1* RNAi. VD and PVM/AVM neurons that are born at L1 seem more sensitive to SMN depletion. The majority of them do not appear in serial imaging. While, PLM/ALM and DDs specially DD3 which are born embryonically seem more resistant to SMN depletion. The variable sensitivity in DDs could be explained by a final maturation process of changing the synaptic pattern of DDs during late L1. As *smn-1* RNAi appearance is under the control of unc-25 and mec-3 drivers with peak of activity at L1, the results suggest that SMN is necessary for terminal neuron differentiation.

We will dentify downstream transcriptomic changes following SMN depletion as well as the identification of the interactomic network of the SMN protein in two different neuronal types to study the molecular pathophysiology of SMA.

Key words : RNA maturaton, Survival of Motor Neuron, transcriptomic, *C. elegans*

700V **Exploring the heat shock transcription factor 1 (HSF-1) mediated transcriptional activation in *Caenorhabditis elegans*** Márton Kovács[1], Dániel Kovács[1], Saqib Ahmed[1], Franciska Szabó[1], Dóra Kiss[1], Tímea Sigmond[1], Tibor Vellai[2,3], Janos Barna[2,-31]Genetics, Eotvos Lorand University, [2]Genetics, Eötvös Loránd University, [3]Genetics Research Group, ELKH-ELTE

HSF1 is an evolutionarily conserved transcription factor - which beyond its function in stress response - plays a significant role in essential biological processes (e.g., development, metabolism, aging) and in certain diseases (neurodegenerative disorders, carcinogenesis, and tumor progression). Despite extensive research the role of HSF1 in these processes is still yet to be fully understood.

In the nematode *C. elegans* there are two known alleles of the *hsf-1* gene: the null mutant *ok600* and the C-terminal transcription activation domain (TAD) deficient *sy441*. While *hsf-1(ok600)* animals show a larval lethal phenotype, *hsf-1(sy441)* worms are viable. Moreover, overexpressing a truncated form of HSF-1 that lacks TAD (HSF-1ΔTAD) enhances the heat stress tolerance of worms. These data suggest that HSF-1 plays a TAD independent role in stress response and in development. Alternatively, HSF-

1ΔTAD preserves some of its transcriptional potential on its own or by interacting with other transcription factors.

We performed an RNA-sequencing analysis and found that surprisingly HSF-1 target genes are activated in *hsf-1(sy441)* mutant background upon robust heat shock. We validated this finding using qPCR and a GFP reporter system. Induction of these target genes is HSF-1 dependent as the depletion of HSF-1 using RNAi abolished the heat induced expression of heat shock protein coding genes in *hsf-1(sy441)* mutant background.

To explore how HSF-1 activates expression of its target genes we are performing forward genetic screens, and to enhance the specificity and efficiency of these screens we are establishing transgenic systems. These screens will possibly lead us to the isolation of novel *hsf-1* mutant alleles, which are viable, but defective in transcriptional activation. We believe that our results would lead to a better understanding of the function of HSF-1.

701V **Differential contributions of condensin to the establishment and maintenance of dosage compensation in *C. elegans*** Jessica Trombley[1], Audry I Rakozy[2], Hend Almunaidi[2], Gyorgyi Csankovszki[21]University of Michigan, [2]Molecular, Cellular, and Developmental Biology, University of Michigan

In organisms with differing numbers of sex chromosomes between sexes, the process of dosage compensation balances X chromosome gene expression. In *C. elegans* hermaphrodites (XX), dosage compensation complex (DCC) binds to the X chromosomes to down-regulate gene expression on both Xs to cause expression levels comparable to males (XO). The SMC protein-containing complex Condensin I^{DC} and additional X chromosome-associated proteins form the complete DCC. As a result of this complex binding to both hermaphrodite X chromosomes, X-specific monomethylation of histone 4 lysine 20 enriches. The DCC also influences the tethering of the X to the nuclear lamina via an unknown mechanism, although the tethering contributes to dosage compensation. Previous studies in our lab showed that these processes contribute significantly to X chromosome structure and gene expression. However, it is unknown if Condensin I^{DC} is needed to maintain dosage compensation in mature C. elegans or whether the additional mechanisms of H4K20me1 and nuclear lamina tethering can maintain repression in the absence of condensin I^{DC}. Null mutations in the DCC lead to varying degrees of hermaphrodite-specific lethality. Yet, the hermaphrodite mutants with defects for the H4K20me1 and nuclear lamina tethering mechanisms are viable. We are testing whether combining defects from the three known pathways will increase dosage compensation defects or destabilize repression during maintenance.

702V **Quantitative analysis of *C. elegans* transcripts by Nanopore direct-cDNA sequencing reveals terminal hairpins in non trans-spliced mRNAs** Denis Dupuy, Florian BernardInserm-Université de Bordeaux

In nematodes and kinetoplastids, mRNA processing involves a *trans*-splicing step through which a short sequence from a snRNP replaces the original 5' end of the primary transcript. It has long been held that 70% of *C. elegans* mRNAs are submitted to trans-splicing. Our recent work suggested that the mechanism is more pervasive but not fully captured by mainstream transcriptome sequencing methods.

In this study, we used Oxford Nanopore's long-read amplification-free sequencing technology to perform a comprehensive analysis of *trans*-splicing in worms.

We demonstrated that spliced leader (SL) sequences presence at the 5' end of the mRNAs affected library preparation and generated sequencing artefacts due to their self-complementarity. Consistent with our previous observations, we found evidence of *trans*-splicing for most genes. However, a subset of genes appears to be only marginally *trans*-spliced. These mRNAs all share the capacity to generate a 5' terminal hairpin structure mimicking the SL structure providing a mechanistic explanation for their non conformity. Altogether, our data provides the most comprehensive quantitative analysis of SL usage to date in *C. elegans.*

703V **Ketamine induces apical extracellular matrix modifications in *Caenorhabditis elegans*** Duygu Yucel[1,21]Genome and Stem Cell Center, Erciyes University, [2]The Institute of Biomedical Engineering, Bogazici University

Ketamine is a widely used anesthetic agent since 1960s and has recently been exploited for its rapid antidepressant action at subanesthetic doses. It has been demonstrated that ketamine induces alterations in extracellular matrix (ECM) in rodent models which in part plays a role in its anti-depressant action. The nematode *Caenorhabditis elegans* serves as a powerful tool for understanding mechanisms of drug action with its short life cycle, genetic amenability and conserved cellular processes. Further investigation is required particularly in *in vivo* systems to gain broader understanding of ketamine's actions. In this study, we aimed to decipher ketamine-mediated alterations using *C. elegans* as a model. We show that ketamine specifically induces apical extracellular matrix modifications (aECM) in the vulva and the cuticle. Ketamine treatment phenocopies neuronal migration and vulval invagination defects of chondroitin mutants despite wild-type like chondroitin staining pattern. Normal vulval expansion and defective vulval eversion phenotypes of ketamine-treated animals are suggestive of alterations in the network

of aECM factors which do not impinge on chondroitin. Ketamine ameliorates impaired movement of a group of *roller* mutants characterised with collagen defects in the cuticle and RNA-seq identifies that 30% of the cuticular collagens are upregulated in response to ketamine. Our findings identified putative novel molecular targets for which the further analysis is underway.

Ketamine alters aECM, neuronal migration and collagen expression in *C. elegans*. We propose *C. elegans* as an animal model to investigate ketamine-mediated ECM modifications.

704V **Reversal frequency as a measure of health in transgenerational longevity mutants** Arthur Colunga, Teresa W LeeBiological Sciences, University of Massachusetts Lowell

Lifespan is a complex trait that can be affected by changes in gene regulation and chromatin structure. For example, in *C. elegans*, mutations in the chromatin modifiers WDR-5 or JHDM-1 cause a transgenerational increase in repressive H3K9me2, which in turn causes a gradual extension of lifespan over many generations. However, it is not clear whether the period of health in these long-lived mutants is also proportionally extended, or whether longevity comes at the cost of extending the period of frailty. To assess health as individuals age, we examined crawling behavior in *wdr-5* and *jhdm-1* mutant populations, both before and after they attained longevity.

While crawling, individuals perform a variety of behaviors, including stereotyped omega-bend reversals, which involve a head-to-tail body contraction. Because reversals require coordination between stimuli, neurons, and muscle tissue, they may act as an indicator of neuromuscular integrity and overall health. Previous studies have found that animals reverse less as they age. Therefore, we hypothesized that our long-lived mutants would reverse more frequently than age-matched wild-type animals. We assessed reversal frequency on days 1, 5, and 8 of adulthood. As previously observed, reversals declined with age in all genotypes. In support of our hypothesis, long-lived *wdr-5* mutants reversed more frequently than wild-type, reflecting an extended healthspan in addition to an extended lifespan. Unexpectedly, long-lived *jhdm-1* mutants reversed less frequently than wild-type in middle-aged and old-aged adults, indicating that behavior may be affected differently in this population. We are currently characterizing whether the difference in reversal frequency changes over generational time.

705V **SEMO-1, a novel copper-dependent methanethiol oxidase and selenium-binding protein, mediates selective stress resistance in *C. elegans*** Verena Alexia Ridolfi[1], Thilo Magnus Philipp[1], Josephine Priebs[1], Anna Patricia Kipp[2], Holger Steinbrenner[1], Lars-Oliver Klotz[11] Friedrich-Schiller-Universität Jena, Institute of Nutritional Sciences, Nutrigenomics Section, Jena, Germany, [2]Friedrich-Schiller-Universität Jena, Institute of Nutritional Sciences, Department of Nutritional Physiology, Jena, Germany

Background: The *C. elegans* ortholog of human selenium-binding protein 1, SEMO1, is a pro-aging factor. SEMO1 deficiency resulted in elevated lifespan and improved resistance against oxidative stress [1,2]. SEMO-1 acts as methanethiol oxidase (MTO), catalyzing the conversion of methanethiol to hydrogen sulfide (H_2S), hydrogen peroxide (H_2O_2) and formaldehyde [2]. Here, we tested the binding of SEMO1 to potential cofactors (copper, selenium) of its MTO activity and whether SEMO-1 is involved in resistance of the worms towards high copper concentrations.

Methods: MTO activity was assessed using a coupled assay based on *in-situ*-generation of methanethiol by a bacterial L-methionine gamma-lyase and detection of the MTO products H_2S and H_2O_2 [3]. Metal binding of recombinant SEMO-1 was detected by total reflection X-ray fluorescence. For stress resistance analyses, N2 wild-type nematodes and a SEMO-1-deficient mutant strain were exposed to Cu(II)-chloride.

Results: Recombinant SEMO-1 binds both copper ions and selenite. Whereas MTO activity in SEMO-1 was largely independent of Se, it was modulated by Cu both *in vitro* and *in vivo*. Addition of Cu(II) or of EDTA to recombinant SEMO-1 enhanced or attenuated MTO activity, respectively. Methanethiol-derived H_2S production in lysates of N2 wild-type worms held on NGM agar supplemented with Cu(II) was increased, whereas addition of the Cu(I) chelator BCS to NGM agar caused an almost complete loss of detectable MTO activity. Given its previously observed protective effect towards high selenite concentrations, we assessed the effect of SEMO1 with respect to Cu exposure of worms. Interestingly, the survival of SEMO1-deficient worms was improved upon exposure to acutely toxic concentrations of Cu(II), whereas they lived shorter than wild-type worms in the presence of chronically toxic copper concentrations.

Conclusions: (1) SEMO-1 binds Cu and Se. (2) SEMO-1 is a Cu-dependent MTO. (3) SEMO-1 mediates selective stress resistance towards Cu, rendering worms susceptible to acutely toxic concentrations while providing protection under conditions of chronic exposure.

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[1] Köhnlein *et al.*, Redox Biol. 28:101323 (2020)

[2] Philipp *et al.*, BioFactors 48:699-706 (2022)

[3] Philipp *et al.*, Redox Biol. 43:101972 (2021)

706V **Modeling human neurodevelopmental disorder-associated *hAGO1* mutations in *C. elegans* Argonaute *ALG-1.*** Ye Duan[1,2], Li Li[3], Ganesh Prabhakar Panzade[3], Amelie Piton[4], Anna Zinovyeva[3], Victor Ambros[51]PMM, UMass Medical School, [2]OEB, Harvard University, [3]Kansas State University, [4]Neurogenetics and Translational Medecine, Institut of Genetics and Molecular and Cellular Biology, [5]PMM, UMass Chan Medical School

MicroRNAs are endogenous non-coding RNAs important for post-transcriptional regulation of gene expression. MicroRNAs associate with Argonaute proteins to bind to the 3' UTR of target genes and confer target repression. Recently, multiple *de novo* coding variants in the human Argonaute gene *AGO1 (hAGO1)* have been reported to cause a neurodevelopmental disorder (NDD) with intellectual disability (ID) and autism-spectrum disorders (ASD). Most of the altered amino acids are conserved between the microRNA-associated Argonautes in *H. Sapiens* and *C. elegans*, suggesting the human *AGO1* mutations could disrupt evolutionarily conserved functions in the microRNA pathway. To investigate how the *hAGO1* mutations may affect microRNA biogenesis and/or functions, we genetically modeled four of the *hAGO1 de novo* variants (referred to as NDD mutations) by introducing the identical mutations to the *C. elegans hAGO1 homolog, alg-1*. This array of mutations caused distinct effects on *C. elegans* microRNA functions, microRNA populations, and downstream gene expression, indicative of profound alterations of microRNA biogenesis, miRISC formation and/or repressive activity. Specifically, we found that the *alg-1* NDD mutations cause allele-specific disruptions in mature microRNA profiles both in terms of overall abundances and association with mutant ALG-1 proteins. Interestingly, we found that the NDD mutations can also be *antimorphic,* wherein the mutant ALG-1 protein interferes with the repressive capacity of paralogous miRISC *in trans* (in this case, miRISC associated with ALG-2). To assess how the *alg-1* NDD mutations modelled in *C. elegans* impact the expression downstream genes, including predicted microRNA targets, we performed ribosome profiling and RNAseq of the mutant worms, and observed global and allele-specific translational perturbations. A large proportion of the perturbed genes are known to be expressed in the *C. elegans* nervous system, and many of them have human homologs whose dysfunction is known to cause NDDs with ID. We anticipate that these cross-clade genetic studies may advance the understanding of fundamental Argonaute functions and provide insights into the conservation of microRNA-mediated post-transcriptional regulatory mechanisms.

707V **Regulation of piRNA-mediated silencing by Arginine Dimethylation** Dylan Wallis[1], Dieu An Nguyen[2], Carolyn Phillips-[21]Biological Sciences, University of Southern California, [2]University of Southern California

RNA silencing is a critically important mechanism through which cells regulate gene expression and protect the genome against aberrant RNAs, transposons, and viruses. This suppression of aberrant transcripts is carried out by evolutionarily conserved small RNA pathways. Small RNAs are loaded onto Argonaute proteins to induce silencing through sequence recognition of specific transcripts through a variety of different silencing mechanisms including post-transcriptional or co-transcriptional silencing. In recent studies, it has been shown that some Argonaute proteins are dimethylated on arginine residues of RG/RGG motifs. To determine whether any *C. elegans* Argonaute proteins are dimethylated, we performed immunoprecipitation and mass spectrometry of the Argonaute proteins containing a high incidence of RG/RGG motifs. Each of the five Argonaute proteins tested contained multiple demethylated arginines. To understand the physiological role of these methylation marks on the Argonaute proteins in the piRNA pathway, we have generated methylation-defective mutants for PRG-1 and HRDE-1. We have found that methylation is not require for localization of PRG-1 and HRDE-1, but loss of methylation on both proteins leads to transgenerational sterility, similar to the null mutant counterparts. We next wanted to investigate whether methylation-defective PRG-1 would be able to re-establish RNAi in animals that have no cellular memory of the *mutator* pathway. Animals with a wild-type copy of PRG-1 are functional to reestablish silencing while *prg-1* mutants cannot; animals carrying the methylation-defective *prg-1* mutant exhibited an intermediate phenotype, further solidifying a defect in the piRNA pathway. Lastly, we sequenced total small RNAs from wild-type and methylation-defective PRG-1 animals; we observed no change in the abundance of piRNAs, but found that the downstream piRNA-dependent 22G RNAs were significantly reduced. These results indicate that PRG-1 methylation plays a key role in linking piRNA-mediated target recognition to downstream 22G RNA amplification and gene silencing. This work has better defined the functional relevance of methylation of Argonaute proteins in the germline, and is the first observation of the role of methylation in the piRNA pathway in *C. elegans*.

708V **JBrowse 2, a new genome browser for WormBase** Scott Cain, Todd Harris, Paulo Nuin, Adam Wright, Lincoln SteinOntario Institute for Cancer Research

Earlier this year, WormBase implemented a new genome browser, JBrowse 2, and removed the Generic Genome Browser (GBrowse), which has been unsupported by the developers for several years. Here we present new functionality provided by JBrowse 2 that is either replacing functionality lost by removing GBrowse, or in many cases, new or improved features over what was available before. These items include dramatically better synteny browsing, evolved scalable vector graphic (SVG) image creation, reimagined user data visualization, and a JBrowse 2 widget that can be embedded in gene and other feature pages. To help users adapt to the new genome browsing interface, we have also created multiple short tutorial videos.

709V **The role of LIN-39 in promoting the longevity of *daf-2* mutant *C. elegans*** Alan Kavsek[1], Lluis Millan-Arino[1], Jerome Salignon[1], Ilke Sen[2], Christian G Riedel[11]Dept of Biosciences and Nutrition, Karolinska Institutet, [2]Dept of Physiology and Pharmacology, Karolinska Institutet

It is well established that the epigenome and nuclear chromatin structure change with age – a phenomenon that has been observed across many species including humans. While the exact relation between these changes and aging remains unclear, studies in *C. elegans* have shown that perturbation of the epigenome can be sufficient to change the rate of aging - indicating at least a partial causal role. One of the most prominent aging-regulatory signaling pathways is insulin/IGF-like signaling (IIS), impairment of which drastically slows aging and extends the organism's lifespan. Here, we explored if also *C. elegans* with reduced IIS experience changes in chromatin organization, and whether this provides new insight into the mechanisms underlying their longevity. Using ATAC sequencing, we found that wild-type and reduced IIS (*daf-2(e1370)*) animals differ in chromatin accessibility at several thousand locations. These differences were predominantly observed in enhancer regions, arguing for their particular importance in conferring *daf-2* mutant phenotypes. Closer inspection of the enhancers revealed that they are enriched for the binding sites of the Hox transcription factor LIN-39 – a known regulator of cellular identity during development. Interestingly, we found that LIN-39 is required for the longevity of *daf-2* mutants and that this role is tissue-specific, taking place mainly in neurons. We are currently identifying the specific neurons involved. Finally, our data shows that LIN-39 regulates aging during development and becomes dispensable during adulthood, providing a prime example of a developmental determinant influencing the aging process.

710V **The mitochondrial genome of *C. elegans* is functionally 6mA methylated** Lantana K Grub[1], James P Held[1], Samantha H Shaffner[1], Tyler J Hansen[2], Marleigh R Canter[1], Maulik R Patel[11]Biological Sciences, Vanderbilt University, [2]Biochemistry, Vanderbilt University

Epigenetic modifications provide powerful molecular means for the transmission of conditional information from parent to progeny. As a maternally inherited genome that encodes essential components of the electron transport chain, the mitochondrial genome (mtDNA) is ideally positioned to serve as a conduit for the trans-generational transmission of metabolic information. We, therefore, set out to establish *Caenorhabditis elegans* as a model to study mtDNA epigenetics. Here we provide evidence that mtDNA of *Caenorhabditis elegans* is methylated. We performed bioinformatic analysis of publicly available SMRT sequencing data and methylated DNA IP (MeDIP) sequencing data, both of which revealed that *C. elegans* mtDNA is adenine methylated at high levels. We further confirmed that mtDNA contains 6mA by leveraging highly specific anti-6mA antibodies. To directly assess mtDNA 6mA, we designed MeDIP assay followed by droplet digital PCR using mtDNA specific primers. This confirmed, with high specificity, that mtDNA is methylated. Combined, these assays provide evidence that supports the presence 6mA in *C. elegans* mtDNA. Additionally, mtDNA methylation can be dynamically regulated and increases in response to the mitochondrial stressor antimycin. This discovery provides an excellent model for future studies to investigate the regulation and inheritance of mitochondrial epigenetics.

711V **Whole-body gene expression atlas of an adult metazoan** Abbas Ghaddar[1], Erick Armingol[2], Chau Huynh[3], Louis Gevirtzman[3], Nathan Lewis[2], Robert Waterston[3], Eyleen O'Rourke[11]University of Virginia, [2]University of California, San Diego, [3]University of Washington

Animals are integrated organ systems composed of interacting cells whose structure and function are in turn defined by their active genes. Understanding what distinguishes physiological and disease states therefore requires systemic knowledge of the gene activities that define the distinct cells that make up an animal. Towards this goal, we present a single-cell resolution transcriptional atlas of a fertile multicellular organism: *Caenorhabditis elegans*. The scRNA-Seq compendium of wild-type young adult *C. elegans* comprises 180 distinct cell types with 18,033 genes expressed across cell types. Fewer than 300 of these genes are housekeeping genes as evidenced by their consistent expression across cell types and conditions, and by their basic and essential functions; 170 of these housekeeping genes are conserved across phyla. The 362 transcription factors with available ChIP-Seq data are linked to patterns of gene expression of different cell types. To identify potential interactions between cell types, we used the *in-silico* tool cell2cell to predict molecular patterns reflecting both known and uncharacterized intercellular interactions across the *C. elegans* body. Finally, we present WormSeq (wormseq.org), a web interface that, among other functions, enables users to query gene expression across cell types, identify cell-type specific and potential housekeeping genes, analyze candidate ligand-receptors mediating communication between cells, and study promiscuous and cell-specific transcription

factors. These datasets, analyses, and tools will enable the generation of testable hypotheses about the cell and organ-specific function of genes in diverse biological contexts.

712A **Neuron type-specific degeneration occurs through distinct mechanisms in a *C. elegans* model of SOD1 ALS** Alexander Lin-Moore[1], Katherine S Yanagi[1], Anne C Hart[21]Brown University, [2]Neuroscience, Brown University

Amyotrophic lateral sclerosis (ALS) is an untreatable, invariably fatal neurodegenerative disease affecting cholinergic and glutamatergic motor neurons. In some patients, initial degeneration occurs in cholinergic neural populations, while in others degeneration begins in glutamatergic populations. Genetic analyses of patients and model systems have found causal mutations in several key genes, but no unifying cellular mechanisms have been identified that explain ALS-associated neurodegeneration, or why affected patients show variation in the site of initial degeneration. Our lab has previously generated a *C. elegans* knock-in model of *sod-1* G85R, a causative allele for heritable ALS, and has leveraged this model to perform the first unbiased genetic screen for suppressors of glutamatergic neurodegeneration in a knock-in ALS model, identifying a series of suppressor lines. While most lines suppress degeneration in both glutamatergic and cholinergic neurons, surprisingly several of these lines show little or no suppression of degeneration in cholinergic motor neurons, representing potential cell type-specific regulators of SOD1 ALS degeneration. The identification of glutamatergic-specific degeneration suppressors presents an opportunity to not only discover cellular pathways driving SOD1 ALS-dependent neurodegeneration, but also to understand the variation in the site of ALS onset.

713A **Stress-induced remodeling of pharyngeal nervous system function via interorgan signaling from the intestine** Surojit Sural, Oliver Hobert Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

The internal state of an animal often acts as a major determinant of its behavioral output. Environmental adversity during early development triggers *C. elegans* to enter the dauer stage during which it displays remarkably distinct behaviors. One such dauer-associated behavior is the complete silencing of the enteric nervous system of the pharynx, which comprises 14 classes of pharyngeal neurons that regulate pumping behavior. Here we show that this silenced state can be reversed via optogenetic stimulation of pharyngeal neurons, thus indicating an active remodeling of the autonomously functioning pharyngeal nervous system in response to environmental stress. We further show that this remodeling of feeding behavior requires activity of the nutritional stress-responsive transcription factor DAF-16/FoxO not just in the pharyngeal neurons, but also non-cell autonomously in the intestine. Moreover, constitutive activation of DAF-16 either in pharyngeal neurons or in the intestine is sufficient to modify feeding behavior even in the absence of nutritional stress. To identify the interorgan signal arising from the intestine that modifies pharyngeal nervous system function, we have performed transcriptional profiling of dauer stage animals with DAF-16 selectively depleted from the intestine using the auxin-inducible degron (AID2) system. These animals fail to remodel their pharyngeal pumping behavior in response to nutritional stress and display misregulated expression of numerous intestinally derived insulin family peptides. We also found that several components of the intestinal secretion machinery are transcriptionally silenced in dauers via DAF-16 activation. Incidentally, blocking intestinal secretion in non-dauers strongly inhibits their pharyngeal pumping behavior, which indicates that an active gut-to-pharyngeal nervous system signal is required to maintain normal feeding behavior in nutrient replete conditions. In summary, our findings highlight the importance of non-neuronal tissues in the remodeling of nervous system function that is required to modify behavioral outputs in non-favorable environments.

714A **Cohesin and a PLZF Protein Direct GABAergic Neuronal Development** Dongyeop Lee, Takashi Hirose, Bob Horvitz HHMI/MIT

Cohesin is a DNA-associated protein complex that regulates diverse cellular processes by altering DNA orientation. The roles of cohesin proteins in sister-chromatid cohesion and meiosis are relatively well-known. However, little is known about the role of cohesin in nervous system development. We have discovered that cohesin and EOR-1, a homolog of the promyelocytic leukemia zinc finger (PLZF) transcription factor, act together to guide specific GABAergic neuron development by preventing those neurons from expressing aspects of other neuronal fates. We performed EMS mutagenesis screens using a *tdc-1p::GFP* reporter, which expresses GFP specifically in the tyraminergic RIM and octopaminergic RIC neurons, and identified mutations in *coh-1* and *eor-2* that cause generation of extra RIM/RIC-like cells. *coh-1* encodes a homolog of RAD21, a subunit of the cohesin complex. *coh-1* mutants have extra RIM-like cells, and inhibition of other cohesin components also caused generation of extra RIM-like cells. The extra RIM-like cells in cohesin mutants are not “undead” sisters of RIM, suggesting that the cohesin complex inhibits fate changes to RIM-like cells rather than promoting the deaths of the sisters of RIM. Using diverse neuronal markers, we showed that the normal identities of the cells that acquire tyraminergic RIM-like traits in cohesin mutants are the GABAergic RMED and RMEV neurons. Our screen also identified *eor-2*, which encodes a co-factor of EOR-1 (PLZF). Similar to cohesin mutants, *eor-1* mutants generate GABAergic RMED/V that express tyraminergic RIM markers. In addition, in cohesin and *eor-1* mutants the normally GABAergic RMED/V neurons fail to express *unc-25*, which encodes a key enzyme in GABA biosynthesis, and have truncated neurites, suggesting that the tyraminergic-like RMED/V lost their GABAergic functions. Interestingly, we found that TRA-4, another homolog of PLZF mediates the generation of the tyraminergic-like RMED/V when cohesin or *eor-1* is genetically

inhibited, indicating that the two PLZF proteins play different roles in the development of the same neurons. We now hope to identify the molecular mechanism by which cohesin and PLZF transcription factors direct neuronal cell-fate determination. No previous interaction has been reported between cohesin and PLZF, and we hope that our experiments will provide novel insights into the functions of the evolutionarily conserved cohesin complex and PLZF transcription factors in animal development.

715A **Building a Homeobox Expression Atlas in the *male C. elegans* nervous system** Robert W Fernandez, Oliver Hobert
Biological Sciences, Columbia University

A central goal in neuroscience is to understand how neural circuits develop, specifically what molecular cues are needed for neuronal identity specification and the proper assembly of individual neurons into neural circuits. Homeobox genes have been extensively implicated in the regulation of the terminally differentiated properties of a mature neuron. Through a homeobox expression atlas in the hermaphrodite *C. elegans* nervous system, we know that every neuronal class is defined by the expression of a unique combination of homeobox genes. Additionally, homeobox genes play a role in neuronal circuit assembly as seen with *unc-42* expression in a synaptically interconnected network involved in locomotory behavior and *ceh-34* regulation of the enteric nervous system. While extensive work has been done for understanding neuronal identity specification and circuit assembly in hermaphrodites, the transcriptional programming for regulation of neuronal identity remains little explored in the male *C. elegans* nervous system. Using available GFP reporter transgenes for homeobox genes and a library of molecular markers, I will test the following concepts in the male *C. elegans* nervous system: 1) Do homeodomain transcription factors regulate the terminal fate features (neurotransmitters and their receptors, neuropeptides) of male-specific neurons? 2) Does every male-specific neuron express a unique combination of homeobox genes that defines the identity of that neuron? 3) Do homeobox genes expressed in synaptically interconnected neurons regulate neuronal circuit assembly? While there is evidence in support of these hypotheses in the hermaphrodite *C. elegans* nervous system, none of these concepts have been tested in the male *C. elegans* nervous system, which is a more complex system due to the addition of 93 male-specific neurons. Preliminary work of 11 homeobox genes has shown expression of several homeobox genes in 51 male-specific neurons as well as expression in sex-shared neurons not present in hermaphrodites. Completion of my work will broaden our understanding of how homeobox genes control the functional properties of a circuit and the assembly of neurons into functional circuits.

716A ***rpm-1*/MYCBP2 interacts with the Kallmann Syndrome gene *kal-1*/KAL1 to regulate neuronal branching** Carlos A. Diaz-Balzac[1], Maria I. Lazaro-Pena[2], Janne Tornberg[3], Jason S Maydan[4], Don Moerman[4], Douglas S. Portman[2], Hannes E. Buelow-[31]Medicine, University of Rochester, [2]Biomedical Genetics, University of Rochester, [3]Genetics, Albert Einstein College of Medicine, [4]Zoology, University of British Columbia

Kallmann syndrome (KS) is characterized by two major deficits, anosmia and hypogonadism. These deficits are the likely result of a neuronal targeting defect of the olfactory axons and a failure of GnRH-secreting neurons to migrate to the hypothalamus. The first gene identified to be associated with this syndrome was KAL1, which encodes a secreted cell adhesion protein named anosmin-1 and is responsible for the X-linked form of KS. Misexpressing the homolog of anosmin-1/KAL1 in *Caenorhabditis elegans* causes a highly penetrant axonal branching phenotype, providing an ideal platform to use forward genetics to identify other genes in the *kal-1* pathway. We identified 2 enhancer mutations that clustered within the same complementation group. Mapping and molecular characterization revealed these mutants to have missense mutations in different domains of the *rpm-1*/MYCBP2 gene. This gene is important for axon termination and synaptogenesis, but has not previously been implicated in the *kal-1* pathway. Interestingly, *rpm-1*/MYCBP2 only enhances *kal-1*-induced branches, as it fails to enhance branches induced by loss of *ttx-3* or *sax-2*. It accomplishes this in a cell-autonomous fashion and requires either more than one known signaling pathway or a novel signaling pathway, downstream of *rpm-1*. We are currently identifying the synaptic targets of the *kal-1*-induced branches and studying the role of *rpm-1*/MYCBP2 in its targeting. Characterization of this novel gene should afford us a deeper understanding of how *kal-1* acts during the development of the nervous system and its role in neural targeting and synaptogenesis. Additionally, *rpm-1*/MYCBP2 may represent a candidate gene for Kallmann Syndrome in humans.

717A **CFI-1 (ARID) and EGL-5 (HOX) transcription factors establish and maintain interneuron identity in a touch reflex circuit** Filipe Alberto Gonçalves Marques, Margaux Marinelli, Paschalis Kratsios Neurobiology, University of Chicago

Touch reflex responses critically rely on the establishment and maintenance of functional circuits composed of mechanosensory, inter- and motor neurons. How these cell types become and remain functional is poorly understood. Here, we report that the molecular identity and function of two interneuron types essential for touch reflex in *C. elegans* is controlled by the combinatorial activity of transcription factors from different families. CFI-1 (ARID3), EGL-5 (Hox), UNC-3 (EBF) and CEH-14 (LHX3) control PVC interneuron identity, while CFI-1 and EGL-5 determine LUA identity. Genetic mutant analysis coupled with ChIP-Seq strongly suggest these proteins act directly to activate transcription of PVC- and LUA-specific effector genes, encoding ion channels, neuropeptides and neurotransmitter biosynthesis components. Further, adult-specific protein depletion experiments revealed a continuous CFI-1 requirement for a proper touch reflex response. Finally, we found that EGL-5 induces CFI-1 expression in both

interneurons, and CFI-1 maintains its own expression through transcriptional autoregulation. Altogether, our study highlights that distinct combinations of transcription factors act as terminal selectors to secure interneuron identity within a touch reflex circuit.

718A **Constructing a tool box for imaging and stimulating pharyngeal neurons to understand foraging behavior in *C. elegans*** Jun Liu[1], Saskia Dirkx[2], Monika Scholz[11]Neural Information Flow, Max Planck Institute for Neurobiology of Behavior – caesar, [2]Life Sciences, HAN University of Applied Sciences

In *C. elegans*, the circuit controlling feeding comprises only 20 neurons and is separate from the 282 somatic neurons, yet it controls food intake and modulates feeding rate effectively. The small pharyngeal circuit is ideally suited to understand the function of a contained, nearly isolated circuit in an intact, behaving animal. Specifically, we want to understand: I) What is the function of individual pharyngeal neurons during foraging? II) How do pharyngeal neurons communicate with the extra-pharyngeal neurons to coordinate foraging behavior? III) What is the activity of individual pharyngeal neurons during foraging? To these ends, we aim to create a toolbox that targets individual and subsets of pharyngeal neurons for expressing a range of optogenetic tools and the genetically-encoded calcium indicator GCaMP.

We use the cGAL and split cGAL system developed by the Sternberg Lab which is adapted from the GAL4-UAS system for the *C. elegans* community. We create driver strains by selecting a single promoter (cGAL) or two intersecting promoters (split cGAL), to achieve specific expression in targeted neuron(s). We then cross these drivers with the effector strains developed by the Sternberg Lab, such as GFP, optogenetic activator/inhibitor and GCaMP, to achieve neuron-specific expression of these proteins. We also create our own effector lines, such as MacQ-mCitrine as a voltage indicator.

To identify promoters that drive unique expression in pharyngeal neurons, we have curated information from the literature and transcription database (CeNGEN). We generate different "promoter::cGAL" driver lines and then verify the expression using the GFP effector lines. Successful candidates are integrated and then crossed to other optogenetic and GCaMP effector lines for targeted neuronal manipulations and calcium imaging of foraging animals.

We have obtained some integrated driver lines (eg: I1, M4, NSM) and are verifying more candidates. We will present our progress in creating such a toolbox. We expect this strain collection to be a valuable tool to understanding the connection between the activity of all neurons in a small circuit and feeding behavior.

719A **Global characterization of neuronal gene expression profiles and neuro-differentiation programs in evolutionary divergent *Caenorhabditis* species** Itai Antoine Toker[1], Eyal Ben-David[2,3], Oliver Hobert[11]Biological Sciences, Columbia University, [2]Department of Biochemistry and Molecular Biology, The Hebrew University of Jerusalem, [3]Present address: Illumina Artificial Intelligence Laboratory, Illumina Inc

The nervous systems of nematodes display a remarkable conservation in the number of neurons and their anatomical arrangement, even when comparing evolutionary distant species. We sought to characterize the molecular facet of how neurons functionally diversify at evolutionary timescales within those anatomical constrains. For this purpose, we profiled single-cell transcriptomes from *C. elegans*, *C. briggsae* & *C. tropicalis* whole-worms, including more than 20,000 differentiated neurons that cover the vast majority of neuronal classes in all three species. We closely examined the cross-species expression dynamics of neuronal terminal identity regulators, neurotransmitter pathways genes and neuropeptidergic signaling genes, because of their potential to elicit functional changes in neural circuits and behavior within an anatomically constrained nervous system. We witnessed pervasive changes in the neuron class-specific expression patterns of homologous neuropeptide-encoding genes between species, reminiscent of the plasticity such neuromodulators display in response to developmental, sex-specific or environmentally-triggered cues in *C. elegans*. More notably, we detected some neuron class-specific changes in the expression of neuronal identity regulators and of neurotransmitter synthesis & transporter genes, which may suggest cases of evolutionary divergence in the core molecular properties of affected neuronal classes and in their synaptic transmission activity. Using CRISPR to fluorescently-tag candidate genes, mutate their regulatory regions and perform cross-species promoter swaps, we are molecularly characterizing the genomic elements and the regulatory logic underlying the observed cases of evolutionary novelty in homologous neuronal classes. By combining our single-cell profiling approach with the genetic accessibility of *Caenorhabditis* nematodes and the accumulated knowledge about neuronal specification in *C. elegans*, we hope to yield insights into the basic principles of brain evolution in recently-diverging species.

720A **Serotonin-Dependent Memory of Juvenile Experience Regulates Sexually Dimorphic Connectivity through the Conserved Zn Finger Transcription Factors LIN-29A** Chien-Po Liao, Maryam Majeed, Oliver HobertBiological Sciences, Columbia University

Sexually dimorphic synaptic connectivity within sex-shared neurons contributes to sexually divergent behaviors, and the dimor-

phic pattern is generated mainly during sexual maturation. However, juvenile experiences can remodel the formation of stereotypically dimorphic connectivity. We find that early juvenile starvation reshapes a male-specific pruning process in PHB>AVA during the later sexual maturation stage via a conserved Zn finger transcription factor LIN-29A in males. Serotonin signaling from a critical time window in early life establishes proper LIN-29A expression in the male sensory neuron PHB upon sexual maturation via G-protein coupled receptor signaling and the CREB transcription factor. We further demonstrate that in the PHB, the Doublesex domain transcription factor, DMD-4, promotes the gene expression of an atypical cadherin protein FMI-1/Flamingo, which is critical to the hermaphrodite-specific growth of PHB>AVA synapses. Upon sexual maturation, LIN-29A inhibits DMD-4 expression and, consequently, *fmi-1* repression, resulting in a failure to grow and maintain PHB>AVA synapses in male animals. In accordance with LIN-29A expression requiring food-dependent 5HT signaling, the LIN-29A -dependent DMD-4 and *fmi-1* expression are derepressed in the male PHB if animals experience juvenile starvation. Lastly, ZNF362, the human ortholog for LIN-29A, rescues the pruning defects in *lin-29a* mutants, suggesting that this function of LIN-29A is evolutionarily conserved. Our findings have revealed that LIN-29A acts as a hub to integrate not only temporal, spatial, and sexual but also experience-dependent information and represses DMD-4-*fmi-1* cassette to promote synaptic pruning in males.

721A **Loss-of-function variants in MYCBP2 cause neurobehavioural phenotypes and corpus callosum defects** Lama AlAbdi[1,2], Muriel Desbois[3], Domnita-Valeria Rusnac[4,5], Artur Kania[6], Ning Zheng[5,7], Brock Grill[3,4,8], Fowzan S. Alkuraya[2]1Department of Zoology, College of Science, King Saud University, [2]Department of Translational Genomics, Center for Genomic Medicine, King Faisal Specialist Hospital and Research Center, [3]CIBR, Seattle Children's Research Institute, [4]Department of Pharmacology, University of Washington School of Medicine, [5]Howard Hughes Medical Institute, [6]Institut de recherches cliniques de Montreal, [7]University of Washington School of Medicine, [8]Department of Pediatrics, University of Washington School of Medicine

The corpus callosum is a bundle of axon fibres that connects the two hemispheres of the brain. Neurodevelopmental disorders that feature dysgenesis of the corpus callosum as a core phenotype offer a valuable window into pathology derived from abnormal axon development. Here, we describe a cohort of eight patients with a neurodevelopmental disorder characterized by a range of deficits including corpus callosum abnormalities, developmental delay, intellectual disability, epilepsy and autistic features. Each patient harboured a distinct *de novo* variant in *MYCBP2*, a gene encoding an atypical Really Interesting New Gene (RING) ubiquitin ligase and signalling hub with evolutionarily conserved functions in axon development.

We used CRISPR/Cas9 gene editing to introduce disease-associated variants into conserved residues in the *Caenorhabditis elegans* MYCBP2 orthologue, RPM-1, and evaluated functional outcomes *in vivo*. Consistent with variable phenotypes in patients with *MYCBP2* variants, *C. elegans* carrying the corresponding human mutations in *rpm-1* displayed axonal and behavioural abnormalities including altered habituation. Furthermore, abnormal axonal accumulation of the autophagy marker LGG-1/LC3 occurred in variants that affect RPM-1 ubiquitin ligase activity. Functional genetic outcomes from anatomical, cell biological and behavioural readouts indicate that *MYCBP2* variants are likely to result in loss of function.

Collectively, our results from multiple human patients and CRISPR gene editing with an *in vivo* animal model support a direct link between *MYCBP2* and a human neurodevelopmental spectrum disorder that we term, *MYCBP2*-related developmental delay with corpus callosum defects (MDCD).

722A **Towards comparative neural physiology of *C. elegans* and *Tardigrada*** Ana M Lyons, Raymond L Dunn, Caroline Mrejen, Saul KatoNeurology, University of California, San Francisco

Researchers have identified specific promoters in *C. elegans* that are active in neurons, allowing them to label, map, and manipulate a diversity of neurons with great precision. Such a molecular toolbox, however, is lacking in sister-taxa such as tardigrades. Like *C. elegans*, tardigrades are interesting candidates for systems-level neuroscientific study due to their microscopic size, transparent bodies, and response to external stimuli. Unlike *C. elegans*, tardigrades possess eight legs, additional sensory organs, and sophisticated limbed locomotory behaviors. Prior work has estimated that tardigrades have several hundred to a few thousand neurons, but the exact number is unknown and the organization of the neuronal network is largely unmapped. Here, we use a series of bioinformatic tools and experimental methods to determine a list of neuron-specific promoters in the tardigrade species *Hypsibius exemplaris*, leveraging the extensive knowledge of neuronal promoters in *C. elegans*. First, we identify a list of putative *H. exemplaris* homologs from a range of known *C. elegans* neuronal genes using BLASTp. We explore the likelihood that these tardigrade homologs may function as neuronal genes by characterizing their GO annotations, using PANNZER2, and functional annotations from InterPro. To further explore the evolutionary conservation of these neuronal genes, we compare sequence similarity of flanking regions and their potential synteny. From this candidate list, we define a list of candidate tardigrade neuronal promoters by taking 100–1000bp upstream of these homologs' start codons. Similarly, we extract 100–1000bp downstream of their stop codons to define a list of putative 3'UTRs. We functionally verify these candidate neuronal promoters and 3'UTRs in vivo by microinjecting adult *H. exemplaris* with extrachromosomal plasmids containing these regulatory sequences and a reporter protein, eGFP or GCaMP6. Expressing these markers in live tardigrades allows for visual-

ization of neuronal populations via confocal microscopy. This pipeline for comparative neuronal promoter discovery allows us not only to map the tardigrade nervous system, but to compare the organization and function of neuronal cell types across evolutionary time.

723A **Decoding the regulatory factors underlying synthesis of toxic dipeptides in a *C. elegans* model of *C9orf72* ALS/FTD** Nidhi Sharma, Kay LaPre, Yoshifumi Sonobe, Raymond P. Roos, Paschalis Kratsios University of Chicago

A hexanucleotide repeat expansion GGGGCC in the non-coding region of *C9orf72* is the most common cause of inherited amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This mutation can lead to neurotoxicity via three non-mutually exclusive mechanisms: 1) loss-of-function due to decreased expression of C9ORF72 protein, 2) RNA toxicity, and 3) proteotoxicity from dipeptide repeat (DPR) proteins. How DPR proteins are synthesized is poorly understood, with only a handful of known regulatory factors necessary for DPRs synthesis, such as the transcription elongation factor Spt4, RNA helicase DDX3X, ribosomal protein eS25 (RPS25), and several eukaryotic translation initiation factors (eIF3F, eIF4B, eIF4H). Our recent study (PMID:34654821) established a *C. elegans* model of *C9orf72* ALS/FTD, characterized by reduced lifespan, motor neuron degeneration, and production of toxic DPRs. With this model, we identified *eif-2D* (ortholog of human eIF2D) involved in the synthesis of PolyGA, one of the most abundant and amyloidogenic DPRs in the brain of *C9orf72* ALS/FTD patients. However, our data also indicated that *eif-2D* does not act alone; *eif-2D* collaborates with other factors to drive PolyGA synthesis. To identify such collaborators, we employ: 1) a **candidate approach** in which we leverage the auxin-inducible degradation (AID) system to deplete *C. elegans* orthologs of two other translation initiation factors *DENR* and *MCTS1*, and 2) an **unbiased approach;** using EMS mutagenesis, we identified mutant strains with reduced PolyGA synthesis. Altogether, our studies aim to identify new factors involved in DPR synthesis.

724A **Principles for coding associative memories in a compact neural network** Christian Oliver Pritz[1,2], Eyal Itskovits[2], Eduard Bokman[2], Rotem Ruach[2], Vladimir Gritsenko[2], Tal Nelken[2], Mai Menasherof[2], Aharon Azulay[2], Alon Zaslaver[21]Neuroscience Institute Cavalieri Ottolenghi, University of Turin, [2]Genetics, Hebrew University Jerusalem

A major goal in neuroscience is to elucidate the principles by which memories are stored in a neural network. Here, we have systematically studied how four types of associative memories (short- and long-term memories, each as positive and negative associations) are encoded within the compact neural network of C. elegans worms. Interestingly, sensory neurons were primarily involved in coding short-term, but not long-term, memories, and individual sensory neurons could be assigned to coding either the conditioned stimulus or the experience valence (or both). Moreover, when considering the collective activity of the sensory neurons, the specific training experiences could be decoded. Interneurons integrated the modulated sensory inputs and a simple linear combination model identified the experience-specific modulated communication routes. The widely-distributed memory suggests that integrated network plasticity, rather than changes to individual neurons, underlies the fine behavioral plasticity. This comprehensive study reveals basic memory-coding principles and highlights the central roles of sensory neurons in memory formation.

725A **The transcription factor MEC-3 regulates the cell surface adhesion proteins FMIL-1 and ZIG-1 to direct neuron-specific synapses in the PVD nociceptive circuit** Tyler Kennedy, Damilola Oje, Kylie Howerter, Barbara O›Brien, Rebecca McWhirter, Jamie Stern, David M Miller Vanderbilt University

Neural circuit architecture is highly reproducible within species, suggesting that genetic programs are key determinants of neuronal wiring diagrams. To investigate this question in *C. elegans*, we used the PVD sensory neuron as a model. PVD drives an escape response to nociceptive stimuli via selective connections with the PVC interneuron in the densely packed ventral nerve cord. We developed fluorescent, live-cell markers for visualizing PVD->PVC synapses and performed an RNAi screen of PVD-enriched transcription factors for PVD synaptic defects. This approach revealed that the LIM homeodomain transcription factor MEC-3 is necessary for PVD->PVC synapses. To identify MEC-3 targets, we used FACS to isolate PVD neurons for RNA sequencing. An RNAi and mutant screen of MEC-3-regulated genes determined that the adhesion proteins FMIL-1 and ZIG-1 phenocopy the PVD miswiring defect in *mec-3* mutants. *fmil-1* (Flamingo-like) encodes an adhesion class GPCR and ZIG-1 belongs to the immunoglobulin superfamily, both of which have been implicated in neuronal target selection in mammalian circuits. Because the PVD nociceptive circuit is established during larval development, we can use our synaptic markers to determine the temporal requirements of *fmil-1* and *zig-1* for PVD->PVC synapses. In particular, we can ask: “Are FMIL-1 and ZIG-1 required for synapse formation or synaptic stability?” questions that are rarely accessible to direct analysis in mammalian neurons.

726A **Impact of the tubulin code on nerve cell morphology & function over long timescales in a living organism** Nagesh Y Kadam[1], Nassima Bouzidi[1], Carsten Janke[2], Wolfgang Keil[11]Institut Curie, CNRS UMR168, [2]Intitut Curie, CNRS UMR3348

Neurons maintain their functions throughout the entire life of an organism by regulating different molecules and adapting to

often complex requirements. Most of these molecules appear as fine-tuning on shorter observation scales, but they may lead to strong impacts on life-long cellular and organism functions. Distinguishing such molecular players and their mechanisms is extremely challenging. Microtubules (MTs) are cytoskeletal polymers, involved in maintaining cell polarity, axon outgrowth and serve as track for transporting different cargoes. Cells modulates their MTs structure, and functions through the expression of different types of alpha and beta tubulin isoforms, and a variety of post-translational modifications (PTMs) enzymes. The tubulin code hypothesis states that the diversity of tubulin isoforms and PTMs assigns unique identities to MTs so that they can perform a large variety of function across various cell types. A growing body of evidence suggests that a small perturbation in tubulin code in naïve or young animals can lead to wide range of age-related neurodegenerative diseases. However, little is known about role of the tubulin code in maintaining MT functions in a spatiotemporal manner *in-vivo*.

Here, we propose to analyze the role of PTMs, more specifically polyglutamylase and deglutamylase enzymes on the morphology and functions of MTs in the mechanosensory neurons of *C. elegans*. The mechanosensory neurons share a unique microtubules composition (15 protofilaments) and are involved in sensing the gentle touch to *C. elegans*. These neurons exhibit aging-induced neurodegeneration, and their microtubules are modified post-translationally via polyglutamylase and deglutamylase enzymes, among other PTMs. The short life cycle of the *C. elegans* and ease of genetic manipulation allows cell-specific gene knock-in's through CRISPR/Cas9, knockdowns with time-dependent depletion of desired proteins through Auxin Inducible degradation (AID) and life-long observations of the resulting phenotypes. We propose long-term *in-vivo* microfluidics imaging of animals harboring different PTMs mutations or time-dependent depletion of specific PTM molecules, analyzing the associated morphological changes and intracellular cargo transport. Our work promises new insights into the role of the tubulin code in maintaining homeostatic functions in neuronal cells over lifelong periods.

727A **The Role of *C. elegans* Metaxins in Mitochondrial Homeostasis** Jonathan V Dietz, Eunchan Park, Nathaly Salazar-Vasquez, Nanci Kane, Carol Nowlen, Christopher Rongo Rutgers University

Mitochondria are critical for neuronal function and health, as they are the primary supplier of energy and calcium storage for neurons. Mitochondrial dynamics – fusion, fission, and motility – facilitate energy production and calcium buffering by mitochondria at specific subcellular sites within neurons. Disturbances in mitochondrial function or dynamics contribute to various neurodegenerative disorders. Using a forward genetic screen in *C. elegans* searching for novel mutants defective in neuronal mitochondrial dynamics, we found that mutations in metaxin 1 (MTX-1), metaxin 2 (MTX-2), and VDAC-1 resulted in fewer mitochondria in *C. elegans* interneuron dendrites. Mammalian metaxin homologs interact with SAM50 to form the sorting and assembly machinery (SAM) complex, which mediates β-barrel protein assembly in the mitochondrial outer membrane (MOM). VDAC-1 is a highly conserved SAM complex substrate that acts as a channel for metabolites across the MOM. We hypothesize that the metaxins promote mitochondrial motility along *C. elegans* interneuron dendrites by mediating assembly of VDAC-1 in the MOM. Mutants for *mtx-1*, *mtx-2*, and *vdac-1* are viable but have reduced lifespans. We found that the mitochondrial unfolded protein response (UPRmt) was activated in *mtx-2* and *vdac-1* mutants, resulting in heat stress resistance and mitohormesis. We are currently investigating the role of *C. elegans* MTX-1 and MTX-2 in MOM β-barrel protein (VDAC-1) assembly and how that impacts neuron integrity.

728A **Characterising the phenomenon of swarming in *C. elegans*** Surabhi Sudevan, Emma Rusconi, Serena Ding Max Planck Institute of Animal Behaviour

Aggregation is one of the best-characterized collective behaviors in *C. elegans*, where the genes, neurons, behavioral rules, and environmental factors involved in the phenomenon are well-known. In this study, I will present my preliminary results characterising a lesser-known collective behaviour of *C. elegans*: swarming. Swarming can be defined as a large, coherent group formed of thousands of worms moving across a bacterial lawn under food-depleting conditions. Under standard laboratory culturing conditions (local density ~ 14.28 worms/cm^2), *npr-1 (ad609)* mutant worms aggregate tightly into groups, but N2 worms are solitary feeders. However, we found that both strains are capable of displaying swarming behavior under certain initial conditions (local density ~ 190.47 worms/cm^2). N2 worms even swarm faster than *npr-1* mutants, and this could be due to the higher feeding rate of N2 worms, which drives faster food depletion, which spatially couples to the displacement of the swarm. Here, I will show preliminary results on the swarm›s behavior, including the movement of the swarm front, worm turnover inside the swarm, and swarm speed.

729A **Giant Ankyrin (UNC-44) mediates neuron maturation through interactions between its C-terminus, UNC-119, and UNC-33.** Matthew S Rich[1], Matthew Labella[1], Sharlei Hsu[1], Sean Merrill[1], Michael J Bastiani[1], Erik M Jorgensen[1,2]1University of Utah, [2]Howard Hughes Medical Institute

Ankyrins are required for maintaining cell morphology by linking membrane-bound proteins to the actin cytoskeleton. Neu-

ronal ankyrins are specialized; a neuron-specific giant isoform is found in all organisms with a nervous system. In *C. elegans*, ankyrin is encoded by *unc-44*, and its giant isoform is 6,994aa long. The C-terminal ~5,000aa of giant ankyrin are specific to the neuronal isoform; most of this sequence is contained in single repetitive exon. We sequenced most extant *unc-44* mutants and found that all were nulls of the giant isoform. Giant ankyrin mutants have highly defective nervous system morphologies with branched and degrading commissures. This phenotype has long been though to be due to a requirement of UNC-44 in outgrowth. We show through longitudinal and live imaging that instead UNC-44 is required for the maintenance of neuron morphology; neuron outgrowth is normal in a giant ankyrin null, but commissures continue to sprout growth cones and eventually degrade.

Giant ankyrin's function in neurons has only recently begun to be clarified: it is required to stabilize microtubules by linking them to the cortical actin cytoskeleton through interactions with spectrin (UNC-70) and other membrane proteins like L1CAM (SAX-7). The structural specifics of how ankyrin binds microtubules is unresolved. Using *in situ* mutagensis with CRISPR/Cas9, we show that the 100 most C-terminal residues of giant ankyrin are both necessary and sufficient for maturation. This function is mediated through interactions with UNC-119 and UNC-33 (CRMP) in a protein complex leading to microtubule stabilization (He et al., 2020). We used AlphaFold to predict the structure of this complex and found that the C-terminus of giant ankyrin folds into a beta sheet that interacts with the beta-sandwich fold of UNC-119. The hydrophobic N-terminus of UNC-33 inserts into the UNC-119 beta-sandwich, occupying a site we thought bound myristoylated N-termini of proteins being trafficked to cilia (Zhang, et al. 2011). Deleting the UNC-33 N-terminus or mutating it to be less hydrophobic fully destroys function, and this function cannot be restored by adding a myristoylation signal. We are currently strengthening our conclusions by assaying the proteins biochemically and structurally. In addition, we are characterizing the conservation of the complex by 'humanizing' it -- replacing UNC-44L, UNC-119, and UNC-33 with their human orthologs. Altogether, these data provide new insights into the roles of UNC-44, UNC-119, and UNC-33 in neuron maturation.

730A **An intestinal sphingolipid promotes neuronal health across generations** Wenyue Wang, Tessa Sherry, Xinran Cheng, Qi Fan, Rebecca Cornell, Jie Liu, Zhicheng Xiao, Roger Pocock Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University

Maternal diet and environment influence the neuronal health of offspring. However, the underlying mechanisms of how maternal nutrition affects neuronal health are unclear. Here, we report that a maternal diet containing ursolic acid (UA) regulates intestinal sphingolipid metabolism to prevent adult-onset axon breaks for two generations. UA is a natural compound found in fruit and is part of a healthy diet. We found that UA protects against axon fragility intergenerationally through increased maternal provisioning of sphingosine-1-phosphate (S1P), a bioactive sphingolipid. Consistent with this result, S1P supplementation mimics UA function in suppressing axon breaks intergenerationally. Mechanistically, UA enhances S1P production by upregulating transcription of the acid ceramidase-1 (*asah-1*) gene in the intestine. S1P is subsequently transferred from the maternal intestine to oocytes to promote intergenerational neuroprotection. Both S1P and UA supplementation improve axon transport and microtubule stability to promote axonal health. Overall, our results demonstrate that UA supplementation in the hermaphrodite diet reduces axon fragility over multiple generations and reveals a novel role of diet-mediated intestinal sphingolipid metabolism in neuronal health.

731A **Molecular screening of genes that regulate the integration of temperature and odor stimuli in *C. elegans*** ZHENHUA SHAO[1,1], KRISTINA GALATSIS[1], YUKI AOKI[1], ASUKA TAKEISHI[21]CBS, RIKEN, [2]CBS/CPR, RIKEN

The natural environment presents organisms with diverse stimuli, and animals' survival depends on making appropriate behavioral choices. Since animal behavior is not solely determined by responses to individual sensory inputs, integration of multiple sensory information in the nervous system is crucial for appropriate responses to environmental stimuli.

To explore how the nervous system integrates multiple information using *C. elegans*, we are investigating the mechanisms of sensory integration of attractive odor and temperature. Studies show worms memorize cultivated temperature (T_c) and migrate toward T_c on a temperature gradient. In addition, worms are attracted by isoamyl alcohol (IAA). We conducted the integration experiments to expose worms to IAA at various environmental temperature and found that migration toward IAA is environmental temperature dependent. We have investigated the possibility of the involvement of several neurotransmitters in the integration of IAA and temperature stimuli. Preliminary we have identified the involvement of dopamine and serotonin signaling in the integration of IAA and temperature stimuli. To further understand the molecular mechanisms of multisensory integration in the nervous system, we have conducted a forward screening and isolated mutants with defects in sensory integration. We expect to provide insights into the neural circuitry and molecular pathways responsible for the integration of multiple sensory inputs in *C. elegans*. by further analyzing the candidate mutants.

732A **Mechanosensory pathways are involved in prey detection in the predatory nematode *Pristionchus***

pacificus Marianne Roca, James LightfootGenetics of Behavior, Max-Planck-Institut für Neurobiologie des Verhaltens – caesar

A remarkable diversity in behaviour is often observed between even closely related species, but there is little understanding for how this variability may emerge and evolve. Novel behavioural traits may arise through the co-option and neofunctionalization of existing pathways or be dependent on the evolution of new regulatory networks. To investigate this, we are exploring the distinct feeding differences between the nematode species *Caenorhabditis elegans* and *Pristionchus pacificus.* Specifically, while *C. elegans* is a microbivorous feeder, *P. pacificus* demonstrates an expansion in its feeding behaviours as they also kill and feed on the larvae of other nematodes. *P. pacificus* predation behaviours are triggered by the predator nose contacting the cuticle of potential prey and previous studies have demonstrated the importance of the sensory cilia for this process. Specifically, *Ppa-daf-19* mutants detect a much lower frequency of larval contacts than wild type animals although a low level of predation is maintained. Hence, mechanosensory and chemosensory systems represent strong candidates for detecting prey and regulating the feeding differences. Genomic analysis of the two mechanochannel gene families, DEG/ENaC and TRP, revealed a striking diversity in the number of mechanosensory genes between these species. Thereafter, we selected 21 candidates of interest and generated CRISPR/Cas9 mutants in each of these to explore any functional and behavioural divergence. Our ongoing preliminary analysis has revealed some genes share a high degree of conservation in function between species including in *mec-3* which is required for the specification of mechanosensory neurons in *C. elegans* and in which the touch response is also nearly abolished in *Ppa-mec-3* mutants. Additionally, the function of *Ppa-mec-4* and *Ppa-mec-10* is generally conserved however minor differences are evident as *Ppa-mec-4* is involved in both gentle touch and harsh touch in *P. pacificus* while only in gentle touch in *C. elegans.* Importantly, these mechanosensory mutants retain their ability to kill other nematodes while double mutants of *Ppa-daf-19; Ppa-mec-10* demonstrate lower predation levels than *Ppa-daf-19* alone. Therefore, predation may depend on the processing of several sensory inputs including both mechanosensation and chemosensation and our ongoing phenotypic analysis will determine the importance of mechanosensation for predation and how these sensory signals have diverged in *P. pacificus*.

733A **'Scanning' and 'glocal search': new behavioral states in worms** Saurabh Thapliyal[1], Isabel Beets[2], Dominique A. Glauser[11]Department of Biology, University of Fribourg, [2]Department of Biology, KU Leuven

Maintaining or shifting between behavioral states according to context is essential for animals to implement fitness-promoting strategies. How integration of internal state, past experience and sensory inputs orchestrate persistent multidimensional behavior changes remains poorly understood. Here, we show that *C. elegans* integrates environment temperature and food availability over different timescales to engage in persistent dwelling, scanning, global or glocal search strategies matching thermoregulatory and feeding needs. Transition between states, in each case, involves regulating multiple processes including the control of AFD or FLP tonic sensory neurons activity, neuropeptide expression and downstream circuit responsiveness. State-specific FLP-6 or FLP-5 neuropeptide signaling acts on a distributed set of inhibitory GPCR(s) to promote scanning or glocal search, respectively, bypassing dopamine and glutamate-dependent behavioral state control. Multisite regulation-dependent behavioral switch by GPCRs in sensory circuits might represent a conserved regulatory logic for persistent behavioral state transitions enabling a flexible prioritization on the valence of multiple inputs.

734A ***Caenorhabditis elegans*** **as a model for human pain genes** Aurore Jordan, Dominique Glauser Biology, University of Fribourg

The detection and avoidance of harmful stimuli are essential animal capabilities. The molecular and cellular mechanisms controlling nociception and its plasticity are conserved, genetically-controlled processes of broad biomedical interest given their relevance to understand and treat pain conditions that represent a major health burden. Recent genome-wide association studies (GWAS) have identified a rich set of polymorphisms related to different pain conditions and pointed to many human pain gene candidates, whose connection to the pain pathways is however often poorly understood. In order to study those conserved pain-related genes, we used a computer-assisted *C. elegans* thermal avoidance analysis pipeline to screen for behavioral defects in a set of 109 mutants for genes orthologous to human pain-related genes. We measured heat-evoked reversal thermosensitivity profiles, as well as spontaneous reversal rate, and compared naïve animals with adapted animals submitted to a series of repeated noxious heat stimuli, which in wild type causes a progressive habituation. Mutations affecting 28 genes displayed defects in at least one of the considered parameters, and could be clustered based on specific phenotypic footprints, such as high-sensitivity mutants, non-adapting mutants or mutants combining multiple defects. Collectively, our data reveal the functional architecture of a network of conserved pain-related genes in *C. elegans* and offer novel entry points for the characterization of poorly understood human pain genes in this genetic model.

735A **Mesodermally-derived GLR glia control *C. elegans* motor behavior and require *let-381/FoxF* and *unc-30/Pitx2* for their fate specification** Nikolaos Stefanakis[1], Yupu Liang[2], Jessica Jiang[1], Shai Shaham[11]The Rockefeller University, [2]Alexion

Glia are cellular components of nearly all nervous systems and are anatomically positioned to affect every aspect of signal transduction and processing. While most glial cells are derived from neuroectodermal precursors, some, like microglia and the glia-like pericytes, which surround blood vessels, are mesodermally derived. Mesodermal glia development is not well understood. *C. elegans* glia can broadly be divided into three classes: 46 sensory-neuron associated glia (sheath and socket cells), four astrocyte-like glia (CEPsh cells), all of which derive from a neuroepithelial lineage, and six mesodermal GLR glia. CEPsh and GLR glia wrap around the exterior and interior aspects of the nerve ring, respectively. While genes affecting the development of *C. elegans* sensory organ and astrocytic CEPsh glia have been characterized, little is known about the development and functions of GLR glia. To understand the molecular basis of GLR glia development, we used FACS followed by RNAseq to identify the GLR transcriptome, and assessed how loss of GLR-enriched transcription factors affects GLR gene expression and morphology. These studies revealed that *let-381/FoxF* and *unc-30/Pitx2* are important regulators of GLR cell-fate specification and differentiation. *let-381/FoxF* has an early role in specifying GLR glia fate and is also continuously required to maintain GLR gene expression by autoregulating its own expression. Using online motif analysis tools, we discovered putative *let-381* binding motifs in the promoters of several GLR expressed genes. Mutagenesis of such motifs disrupts endogenous GLR gene expression, suggesting that *let-381* directly controls GLR molecular identity. *unc-30/Pitx2* acts downstream of *let-381* to control GLR glia morphology and to repress gene expression of another mesodermal cell, the head mesodermal cell (HMC), in GLR glia. Together, *let-381* and *unc-30* are sufficient to induce GLR gene expression when ectopically expressed. We used the transcription factor mutants we identified to explore functions of GLR glia, whose anatomy and gene expression profile suggest involvement in motor behavior. Remarkably, GLR-defective animals display various motor behavior defects including increased reversal frequency and locomotory pausing. Loss of CEPsh glia is also associated with certain motor behavior defects, suggesting that both glial sets control nerve ring activity. We are pursuing candidate gene approaches to understand the molecular mechanisms through which GLR glia control these behaviors.

736A **Using *Caenorhabditis elegans* to understand the mechanism and the function of towering behaviour** Daniela Malgarini Perez[1,2], Thomas Stier[1], Serena Ding[11]Max Planck Institute of Animal Behavior, [2]Max Planck of Animal Behavior

The versatile model organism, *Caenorhabditis elegans*, exhibits various collective behaviours such aggregation, swarming, dynamical network formation, and towering. Although some of these behaviours have been known for decades, comprehensive quantitative studies have only begun more recently and have thus far been restricted to 2D behaviours. In particular, 3D behaviours such as towering (collective nictation) are not only fascinating to observe but also critical for the ecology in a wide range of nematode species, as it is understood to be a dispersal mechanism integral to their boom-and-bust lifestyle. However, a detailed characterisation of the behaviour and its adaptive value is still missing. In this study, we identify experimental conditions to produce worm towers in the lab and address three fundamental questions on towering behaviour using *C. elegans*: (1) Do individuals at the top of the tower have higher reproductive fitness (produce larger brood) than individuals at the bottom or outside the tower? (2) Do worms of all life stages present towering behaviour? (3) Does the presence of a vector (*Drosophila* sp) induce stronger towering? We found that we could reliably promote towering under laboratory conditions by providing vertical structures and exposing a high density of worms (approximately 1000 individuals) to starvation. By developing an imaging-based high throughput method for counting brood sizes using multi-well plates and automated worm tracking, we did not find a strong pattern of reproductive fitness between worms that occupy distinct parts of the tower. Worm of all life stages can present towering behaviour including mixed life stages, forming towers of up to 500 individuals. Dauers take longer to form towers indicating that, as a dispersal life stage, individuals explore the environment on the surface level before climbing on structures. Lastly, contrary to our predictions, worms tower regardless of the presence of a vector, indicating that food depletion alone is sufficient to promote towering. These results lay the necessary foundation for directly addressing our fundamental questions on the mechanism and function of towering behaviour in the future.

737A **Functional and behavioral impacts of human *NRXN1* variants identified through expression in *C. elegans* neurons.** Dustin Haskell[1], Mara Cowen[1], Michael Hart[21]Genetics, University of Pennsylvania Perelman School of Medicine, [2]University of Pennsylvania Perelman School of Medicine

Neurodevelopmental and neuropsychiatric conditions are genetically and phenotypically complex, making *in vivo* mechanistic studies difficult. Neurexins are conserved synaptic adhesion proteins that are critical to neuronal development and function. Genetic perturbations in human *NRXN1* have been identified in patients with autism spectrum, schizophrenia, and Tourette's syndrome. To better understand the mechanistic and behavioral roles of neurexins, we are using *C. elegans* as a model to uncover the functional impact of disease-associated variations within the *NRXN1* gene. The human *NRXN1* gene produces 3 major isoforms (alpha, beta, and gamma) and undergoes significant alternative splicing, both of which contribute to a large and diverse repertoire of NRXN1 proteins. We initially set out to characterize 8 isoforms of human *NRXN1* identified from control (4 control isoforms) or schizophrenia proband hiPSCs which harbor 3' *NRXN1* deletion (4 mutant isoforms). We generated transgenic strains that express each human *NRXN1* isoform in all neurons in (*nrx-1(wy778)*) mutants, which lack both isoforms of the singular *C. elegans* neurexin ortholog (*nrx-1*). Furthermore, ongoing work seeks to generate several CRISPR strains that insert *NRXN1* isoforms

into the endogenous *nrx-1* loci (in a *nrx-1* null background), to better understand the impact of the endogenous promoter and 3›UTR on their expression. Additionally, we characterized the impact of a conserved missense mutation identified in an autism proband (L18Q) in the *nrx-1* gene via CRISPR/Cas9 modification (L16Q), and also expressed *nrx-1* in all neurons with and without this mutation. For each *NRXN1* isoform and the conserved *nrx-1* variant, we assayed protein expression, localization, neuron morphology, and impact on behavior. We find that variants in *C. elegans nrx-1* alter multiple foraging related behaviors. Similarly, the disease-specific isoforms of human *NRXN1* show altered expression/localization in neurons and alter neuron morphology and behavioral phenotypes when compared to control isoforms in *C. elegans*. Ongoing work includes in-depth characterization of these *NRXN1* variants to fully understand the mechanistic role neurexin plays in neurodevelopmental changes. This work provides a convenient and tractable model to screen for functional conservation of genes and functional impact of disease-associated variants in a reasonably high-throughput fashion.

738A **Characterisation of a Cationic Dopamine-Gated Ion Channel in *C. elegans* and other invertebrates** Amy Courtney[1], Iris Hardege[1], Ruth Styfhals[2], Milena Marinkovic[3], Gáspár Jékely[3], Eduardo Almansa[4], Eve Seuntjens[2], William Schafer[1,21]MRC Laboratory of Molecular Biology, [2]KU Leuven, [3]University of Exeter, [4]Instituto Español de Oceanografía

Cys-loop ligand-gated ion channels (LGCs) are essential for fast neurotransmission. Vertebrate LGCs include excitatory ACh/5HT3 receptors as well as inhibitory GABA/glycine receptors. However, invertebrate LGCs have diversified significantly, with *C. elegans* having channels activated by novel ligands such as protons, betaine, tyramine, octopamine, histamine and choline as well as channels with “inverted” ion selectivity (excitatory GABA channels and inhibitory ACh channels). However, our understanding of invertebrate LGC function has been restricted to the major model organisms, and the true functional diversity of these receptors is not understood. We addressed this gap by exploring the function of LGCs from a previously neglected invertebrate phyla. We performed phylogenetic analysis on metazoan LGCs and identified LGCs with potentially novel ligands in multiple invertebrate species, including the cephalopod mollusc *Octopus vulgaris*. We performed deorphanization experiments on octopus LGCs using *Xenopus* oocytes and identified the first example of an excitatory dopamine-gated channel in any species. The phylogenetic analysis also revealed that this channel is present in other molluscs, nematodes, annelids, some arthropods and is missing in platyhelminths and vertebrates. Oocyte experiments showed that *C. elegans* also possesses this channel, but unlike the other species which have a homomeric version, worms have a heteromeric version. We now understand the pharmacology and evolutionary history of this channel, but we are also keen to understand its role *in vivo*. There are 12 dopaminergic neurons in *C. elegans*. Combining connectomic data and expression data we identified many neurons which express this dopamine-gated channel and are synaptically connected to dopamine synthesising neurons, the top candidates include RMD, RMG, and RMH. In-depth behavioural, optogenetic and calcium imaging experiments are ongoing to uncover whether dopamine is routinely used as a ‘fast neurotransmitter’ at synapses in *C. elegans*. This will challenge the long-held assumption that dopamine acts primarily as an extrasynaptic signalling molecule in all nervous systems.

739A **Studying evidence accumulation and the neurogenic control of pharyngeal pumping** Luis Alvarez[1], Jun Liu[1], Citlali P. Campos[2], Richard W Yan[2], Elizabeth M. C. Hillman[2], Monika Scholz[31]Neural Information Flow, Max Planck Insitute Neurobiology of Behavior, [2]Columbia University, [3]Max Planck Insitute Neurobiology of Behavior

During motor tasks, animals continually integrate sensory information about the environment to make informed decisions. The nematode *Caenorhabditis elegans* acquires food by the pumping action of its pharynx: a neuromuscular organ that is controlled by a local network comprising 20 neurons of the total of 302 neurons in the animal. Cycles of pharyngeal muscle contractions and relaxations result in the acquisition of bacteria. Upon contraction, the surrounding fluid and the bacteria therein are sucked into the lumen of the pharynx. Upon relaxation, fluid is expelled while bacteria are sequestered in the animal. This pumping motion requires energy expenditure and thus, it only pays off when enough bacteria are ingested. Additionally, it comes to a risk as it exposes the digestive tract of the worm to potentially noxious substances. Previous studies have suggested a decision-making process that allows the animal to optimally balance the pumping activity to acquire food while reducing energy expenditure and the animal›s exposure. This work suggests that worms sample their surroundings by stochastic pumping. Based on the food acquired during sampling, worms adjust their pumping rate accordingly. We are interested in identifying the neurons, signals, and transfer functions in the worm that enable this decision-making process. We record worms exposed to different levels of food using a combination of microfluidics and SCAPE microscopy. SCAPE is a light-sheet microscopy method that allows for whole-brain imaging in 3D at high volume rates with cellular resolution and low phototoxicity. We will show our advances in using these techniques to image neural activity of *C. elegans* during feeding.

740A **Bidirectional spiraling is a novel collective behavior in C. elegans involving the AFD neuron** Laura Persson[1], Marlena Rohde[2], Noelle L’Etoile[31]Cell and Tissue Biology, University of California, San Francisco, [2]University of California San Francisco, [3]Cell and Tissue Biology, University of California San Francisco

Many organisms engage in collective behaviors to benefit the survival of the individual. When many individuals simultaneous-

ly follow a set of behavioral rules about how to interact with one another and their environment, group behaviors with new functionalities can emerge. This phenomenon of emergent behavior occurs widely in nature, from prokaryotes to honey bees to herd animals. Remarkably, collectives can show complex, high level organization in the absence of centralized control, a concept coined "swarm intelligence". While this concept has been widely employed in computer science[1], our ability to build biological systems with emergent properties or to perturb biological collectives in predictable ways is limited. The study of emergent behaviors in biology has been hampered by the experimental intractability of most organisms known for complex group behaviors. Here, we report the discovery of an emergent group behavior in the highly tractable model organism *C. elegans*. At sufficient animal densities, a simple environmental cue prompts *C. elegans* to initiate a highly coordinated bidirectional spiral that condenses the population at the center of their former distribution. Notably, we find that isolated animals do not respond in the same way to the cue indicating that this behavior is emergent. Moreover, the presence of a nearby but physically separate high density population can elicit spiral-like behavior from isolated individuals in response to the cue suggesting that inter-worm communication can occur "wirelessly" or without direct interactions between individuals. We find that mutants defective in the function of the AFD neuron also show defects in spiraling behavior, and that the AFD neuron is depolarized in response to the cue that prompts spiraling. We are exploring a potentially novel role for AFD in orchestrating group behavior through a combination of volatile-based long-range signaling and local worm-to-worm communication between neighbors. We are additionally exploring the possibility that group spiraling is an efficient strategy for gathering dispersed individuals across distances many times the size of the organism.

741A **Effects of early life adversity on the adult brain of *Caenorhabditis elegans*** Giulio Valperga, Oliver Hobert Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

Early life adversity can permanently alter an individual's behaviour, metabolism and physiology later in life. In neurons, this process of biological embedding leads to life-long epigenetic and gene expression changes, but its regulatory logic is poorly understood. When exposed to aversive conditions during early-life, *C. elegans* enters a developmental arrest stage ("dauer") which it exits when conditions return favourable. Passage through the dauer-stage leaves notable physiological, behavioural and gene expression changes in adult individuals. To investigate the molecular details that establish and maintain alterations in response to early-life adversity, we undertook an ongoing effort to characterise nervous-system-wide changes in neuropeptide gene expression and behavioural programs in post-dauer animals. Using an extensive collection of CRISPR/Cas9-edited, endogenous reporter alleles and together with the NeuroPAL landmark strain, we discovered several neuropeptide genes with robust gene expression changes in post-dauer animals. Alterations ranged from elevated or diminished expression to entirely new expression in distinct neural types. For example, *nlp-12* showed heightened levels in the DVA neuron in post-dauer individuals, while *trh-1* showed expression in the neurosecretory pharyngeal neuron NSM in post-dauer animals but not in controls. For a subset of these reporters, we examined post-dauer from both sexes and identified dimorphic regulation. For instance, *flp-32* showed a striking up-regulation in PHC exclusively in post-dauer hermaphrodites. Taken together, these data provide useful tools to uncover the regulatory logic controlling the establishment and maintenance of life-long gene expression changes in response to early-life adversity.

742A **Visualizing neuronal outgrowth and circuit assembly of the *C. elegans* pharyngeal nervous system** Wen Xi Cao, Oliver HobertBiological Sciences, Columbia University

The pharyngeal nervous system of *C. elegans* comprises 20 neurons of 14 anatomical classes. During embryogenesis, this simple and primitive nervous system develops in isolation within the feeding organ of the animal's pharynx. In contrast to the rest of the *C. elegans* neurons, these pharyngeal neurons are embedded within the pharyngeal muscles, highly interconnected to each other, and function autonomously from the main nervous system in many ways which parallel enteric nervous systems across metazoans. While many mechanisms of axon guidance and circuit assembly have been uncovered over the last several decades, their function in the development of pharyngeal neuron circuits has not been well-characterized. Here, we aim to use a combination of live imaging, electron microscopy (EM) and mutant analysis to fully interrogate mechanisms that regulate growth and development of the pharyngeal nervous system.

Using previously reported gene expression patterns and recent neuron-enriched single-cell sequencing data (CeNGEN), we have built a toolkit of neuron class-specific and early embryonically expressed reporters to visualize the growth and circuit assembly for nearly all 14 classes of pharyngeal neurons throughout embryogenesis, as well as non-neuronal pharyngeal tissues, including muscles and glands. With this reporter toolkit, we make use of dual-view Inverted Selective Plane Illumination Microscopy (diSPIM), which enables live imaging with spatially isotropic and high temporal resolution of neurite growth in the developing embryo. By combining multiple colors on the diSPIM and with reference to EM snapshots that provide whole tissue context, we find stereotyped patterns in the timing and morphology of neurite growth. We are currently working to wholly characterize the growth and assembly of the pharyngeal nervous system during embryogenesis. Furthermore, known mechanisms of neurite outgrowth and circuit assembly in *C. elegans*, such as axon guidance and cell adhesion molecules, also play previously

underappreciated roles in pharyngeal neurons. Combining these visualization tools with mutant analyses will provide a comprehensive study of the processes of pharyngeal circuit formation. We aim to uncover the underlying mechanisms involved in the formation of the *C. elegans* pharyngeal nervous system as a model not only for enteric nervous systems across animals, but also fundamental principles of neuronal circuit growth and assembly as a whole.

743A **AGEF-1 activates RAB-35 and promotes axonal fragility by altering neuron-epidermal attachment** Igor Bonacossa-Pereira, Sean Coakley, Massimo HilliardThe University of Queensland

It's a hard knock life, and consequently all tissues must be robust enough to maintain their integrity and shape. Whilst neurons display a delicate polarized morphology, they are in fact robust, mechanically resilient cells capable of resisting strain throughout the life of an organism. In healthy individuals, the integrity of a neuron's longest neurite, the axon, is preserved despite experiencing chronic mechanical stress due to body movement. This indicates the existence of specialized protective mechanisms, which may lead to axonal degenerative phenotypes when disrupted. In living organisms, sensory neurons are embedded and ensheathed within epithelia, and it is poorly understood how this tissue contributes to maintain axonal integrity. Using *C. elegans* as a model system, we have revealed that within the skin, the membrane-associated cytoskeletal spectrin network functions in synergy with the small GTPase RAB-35 to stabilize neuron-epidermal attachment structures in response to mechanical strain, protecting the axons of touch-receptor neurons embedded in this tissue against movement-induced damage. In this context, RAB-35 activation induces axonal damage. Here, through an unbiased forward genetic screen we have identified a GTP exchange factor (GEF), AGEF-1, previously associated with the endocytic-recycling machinery, that impacts axonal maintenance. We show that AGEF-1 functions non-cell-autonomously within the skin to promote RAB-35-induced axonal damage. Next, we demonstrate that the function of this GEF is highly conserved, with expression of its human ortholog ARFGEF2/BIG2 able to promote axonal damage. We propose that AGEF-1 is a novel activator of RAB-35 and promotes axonal damage by modulating RAB-35 activity. Taken together, our data supports a model where the skin fine-tunes the maintenance of touch-receptor neurons by controlling cell-cell adhesion.

744A **Membrane calcium ATPase-3 (MCA-3) is a Calcium/calmodulin-dependent protein kinase-1 (CMK-1) target essential for *C. elegans* thermo-nociceptive habituation** Martina Rudgalvyte, Zehan Hu, Dieter Kressler, Joern Dengjel, Dominique A Glauser University of Fribourg

Nociception is a self-protection system alerting animals of potential damage and serving as a foundation for different forms of pain in human. Some chronic pain conditions may arise from defective modulation in the nociceptive pathway, including within nociceptors, the primary nociceptive sensory neurons. Sensory plasticity is essential for animals to survive and adapt to changing environment. This pathway appears to be highly conservative in many species. We use *Caenorhabditis elegans* as a model due to its ability to detect noxious stimuli, perform avoidance behaviors in the form of stimulus-evoked reversals and habituate to repeated stimuli causing a desensitized, analgesia-like state. Calcium-calmodulin-dependent protein kinase 1 (CMK-1) mediates cellular responses to increased calcium levels and is crucial in nociceptors for this avoidance behavior plasticity. However, the downstream elements of the CMK-1 pathway remain unclear.

The worm ortholog of mammalian plasma membrane ATPase ATP2B3, MCA-3 (membrane calcium ATPase) is essential component of calcium homeostasis in the cell. We identified this protein as a potential downstream phospho-target of CMK-1 using *in vitro* CMK-1 kinase assays on both peptide and protein from total worm isolates via shotgun phosphoproteomics. We found that *mca-3* loss-of-function mutants failed to habituate in response to repeated noxious heat stimuli. Furthermore, epistasis analysis results indicate that *mca-3* is required for a *cmk-1* gain-of-function mutation to enhance habituation. Collectively, these results suggest that the CMK-1 pathway might regulate nociceptive habituation by controlling cell calcium homeostasis via the plasma membrane calcium pump MCA-3. We are currently working at determining the locus of action of MCA-3.

745A **Neuropeptidergic signaling underlying experience-dependent behavioral plasticity** Ellen Geens[1], Hanna Schön[2], Sara Van Damme[1], Sandeep Venkatraman[1], Giulio Valperga[3], Mario de Bono[2], Isabel Beets[11]KU Leuven, [2]Institute of Science and Technology Austria, [3]Columbia University

When navigating their environment, animals are confronted with numerous sensory cues. In addition to acute responses, neurons adapt to sensory experience by long-lasting changes in gene expression. These may influence behavioral responses to one specific stimulus, but often also affect other sensory circuits, resulting in sensory cross-modulation. Given its evolutionary benefit, it is not surprising that experience-dependent behavioral plasticity is found across metazoans. However, the molecular and cellular mechanisms driving sensory crossmodulation remain largely unknown.

In *C. elegans*, prolonged exposure to aversive oxygen (O_2) levels changes O_2-escape behavior as well as responses to other aversive cues. To dissect the molecular pathways that underpin these behavioral changes, we used cell-type-specific RNA sequencing to identify genes that are differentially expressed in the O_2-sensing neurons in animals grown at either high (21%) or low (7%)

O_2. Within the group of O_2-regulated genes, we find that neuropeptide genes are omnipresent. Expression analysis of reporter-tagged endogenous genes confirmed experience-dependent expression of neuropeptide genes in the O_2-sensing neurons. To gain insight into the peptidergic circuits regulated by O_2 experience, we identified the receptors for differentially expressed neuropeptides using a reverse pharmacology approach. We found evolutionarily conserved pathways among the identified neuropeptide-receptor couples, including the bilaterian somatostatin signaling system. To uncover the biological functions of experience-dependent neuropeptide signaling, we are investigating its effects on the plasticity of O_2-escape behavior and responses to other aversive stimuli. This will provide further insight into the experience-dependent signaling mechanisms driving behavioral plasticity and sensory cross-modulation.

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746A **Behavioural analysis of nematode natural diversity** Tanara Peres[1], Erik Andersen[2], Kathryn A. Hodgins[3], Andre Brown-[41]Institute of Clinical Sciences, Imperial College London, [2]Northwestern University, [3]Monash University, [4]Imperial College London

Convergent evolution of behaviours occurs when different species of animals independently acquire similar behavioural strategies, such as different species of Hawaiian spiders spinning similar web architectures. Is this convergence due to changes in the same genes or does evolution act through distinct genetic routes to create the same behaviours (repeated evolution)? To answer this, we will use *Caenorhabditis* natural variation to compare behavioural patterns to whole-genome genotype data. We analysed the behaviour of wild isolate strains of three different species (*C. elegans*, *C. briggsae* and *C. tropicalis*). We used the 12 most representative strains of each species, including the divergent set from the *C. elegans* Natural Diversity Resource (CeNDR) collection. Age synchronized adult worms were pipetted onto 96 well plates seeded with OP50 bacteria on peptone free NGM. Worm behaviour was recorded using high-resolution multiworm tracking, before, during and after a blue light stimulus. The camera used to track worms is called Hydra, which contains five rigs. Each rig is composed of six 12-megapixel cameras with sufficient resolution to estimate the pose of *C. elegans* worms. Tracking data was analysed using Tierpsy Tracker software. PCA analysis of preliminary data shows *C. tropicalis* cluster separately from *C. elegans* and *C. briggsae*. Next, we will analyse 200 additional strains and obtain behavioural fingerprints from these animals, compare their genome and connect behavioural differences to genetic differences. Combining these data, we will determine whether a behavioural pattern is a result of repeated evolution or convergent evolution.

747A **Molecular mechanisms of *Bacillus subtilis*-induced protection against □-synuclein aggregation in *Caenorhabditis elegans*** Deep Prakash[1], Maria Eugenia Goya[1,2], Samanta Paz Recio[1], Tom Humphreys[1], Charlotte Crawford[1], Magda Olech[1], Feng Xue[1], Liesa Salzer[3], Johana Jarkulischová[2], Martin A. Schepers[2], Stefan Busscher[2], Kevin S. Chen[4], Kazuko Fujisawa[4], Michael Witting[3], Lorraine V. Kalia[4], Ellen Nollen[2], Maria Doitsidou[11]University of Edinburgh, UK, [2]ERIBA, UMCG, University of Groningen, The Netherlands, [3]Research Unit Analytical BioGeoChemistry,Helmholtz Zentrum, Germany, [4]Krembil Brain Institute, Toronto Western Hospital, University Health Network, Toronto, Canada

Parkinson's disease (PD) is the second most common neurodegenerative condition. Accumulation of □-synuclein (□-syn) protein is one of the hallmarks of Parkinson's disease (PD) and central to the pathogenesis. Growing evidence suggests that gut microbiota plays an important role in the progression and severity of the condition. Notable differences have been reported in the gut microbiota composition of PD patients compared to healthy controls. However, how gut bacteria affect PD pathology remains unclear.

We used a well-characterized *C. elegans* model that expresses human □-syn fused to yellow fluorescent protein in muscle cells (Van Ham et al, 2008). When this transgenic strain is fed with a laboratory diet of *Escherichia coli* OP50, α-syn inclusions are formed, visible with fluorescence microscopy. In a screen testing the effect of different bacterial diets on □-syn inclusions, we previously showed that *B. 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 affects α-syn associated neurodegeneration and behavioral phenotypes.

Several *C. elegans* metabolic pathways are differentially regulated when fed with *B. subtilis,* of which alterations in the sphingolipid metabolism pathway partially contribute to the anti-aggregating effect. However, the identity of the genetic regulators in *B. subtilis* and the bacterial metabolite(s) modulating the protective effect remain elusive.

To identify the genes in *B. subtilis* crucial for its protective effect we screened a genome reduction library of non-essential genes of *B. subtilis* 168 (Tanaka et al, 2013), which collectively covers 76 percent of the genome. Concurrently, we are also undertaking a genome-wide screen using a *B. subtilis* single gene deletion library (Koo et al,2017). Our results so far suggest that various bacterial components are involved in modulating α-syn aggregation. Among promising candidates are genes involved in the TCA cycle, ABC transporter groups and nucleotide metabolism associated genetic pathways. Our current efforts are directed towards

dissecting these pathways to find molecular and mechanistic bases of the protective effect. In a complementary approach, we use comparative metabolomics to identify potentially protective metabolite(s). Overall, our study has the potential to reveal compounds with disease-modifying potential for PD.

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748A **Fatty acids derived from the probiotic *Lacticaseibacillus rhamnosus* HA-114 suppress age-dependent neurodegeneration** Audrey Labarre[1,2], Ericka Guitard[1,2], Gilles Tossing[1,2], Anik Forest[3], Eric Bareke[1], Marjorie Labrecque[1,4], Martine Tétreault[1,2], Matthieu Ruiz[3,5], J Alex Parker[1,21]CRCHUM, University of Montreal, [2]Department of neurosciences, University of Montreal, [3]Research Centre, Montreal Heart Institute, [4]Department of biochemistry, University of Montreal, [5]Department of nutrition, University of Montreal

The human microbiota is believed to influence health. Microbiome dysbiosis may be linked to neurological conditions like Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. We report the ability of a probiotic bacterial strain in halting neurodegeneration phenotypes. We show that *Lacticaseibacillus rhamnosus* HA-114 is neuroprotective in *C. elegans* models of amyotrophic lateral sclerosis and Huntington's disease. Our results show that neuroprotection from *L. rhamnosus* HA-114 is unique from other *L. rhamnosus* strains and resides in its fatty acid content. Neuroprotection by *L. rhamnosus* HA-114 requires *acdh-1/ACADSB*, *kat-1/ACAT1* and *elo-6/ELOVL3/6*, which are associated with fatty acid metabolism and mitochondrial β-oxidation. Our data suggest that disrupted lipid metabolism contributes to neurodegeneration and that dietary intervention with *L. rhamnosus* HA-114 restores lipid homeostasis and energy balance through mitochondrial β-oxidation. Our findings encourage the exploration of *L. rhamnosus HA-114* derived interventions to modify the progression of neurodegenerative diseases.

749A **Loss of peptidergic regulation of cholinergic transmission induces postsynaptic homeostatic compensation** Jiajie Shao, Jana Liewald, Alexander GottschalkBuchmann Institute for Molecular Life Sciences, Goethe University

Neural homeostasis requires proper communication between pre- and postsynaptic cells. Different mechanisms are proposed to modulate this signaling including transsynaptic adhesion molecules and, to some extent, retrograde signaling. We propose that also neuropeptidergic signaling may regulate this synaptic homeostasis. In this study, we investigated peptidergic signaling in the context of cholinergic transmission at the neuromuscular junction (NMJ). We have shown previously that neuropeptide (NP) signals regulate the cholinergic output at the level of synaptic vesicle (SV) filling [1]. Starting from analyzing the dense core vesicle release deficient mutant *unc-31*, we observed reduced acetylcholine transmission by electrophysiological recording of body muscle EPSCs. However, unexpectedly, we observed enhanced muscle contraction and Ca^{2+} influx compared to wild-type animals following optogenetic activation of cholinergic motor neurons. We propose that in response to reduced cholinergic transmission, postsynaptic homeostasis could compensate for the reduced input and thus maintain synaptic strength. To test this idea, we directly activated the muscles with channelrhodopsin-2 in wild-type and *unc-31* animals. Muscle activity (evoked muscle contraction) was significantly increased in *unc-31* animals. This phenotype was rescued by *unc-31* cDNA expression specifically in GABAergic, but not cholinergic neurons, indicating a complex peptidergic interplay between the two classes of motor neurons. A mutant of the proprotein convertase *aex-5/kpc-3* phenocopied *unc-31*, indicating that specific classes of NPs are responsible for this homeostasis. Furthermore, we identified the L-type voltage gated Ca^{2+} channel EGL-19 as a modulator in muscle cells mediating the postsynaptic homeostatic response. We currently work on identifying the released peptides from GABAergic neurons and to understand the underlying mechanisms of how muscle homeostasis is achieved.

1. Steuer Costa et al. (2017) Curr Biol 27, 495-507.

750A **Restructuring of a lateralized neural circuit during associative learning by asymmetric insulin signaling** Leo T. H. Tang[1], Garrett A. Lee[2], Steven J. Cook[2], Jacqulin Ho[1], Cassandra C. Potter[1], Hannes E. Buelow[1,21]Genetics, Albert Einstein College of Medicine, [2]Neuroscience, Albert Einstein College of Medicine

Vertebrate and invertebrate brains appear largely bilaterally symmetric, yet display clear functional, molecular, and more subtle structural asymmetries. Lateralized brain functionality is plastic and has been implicated in learning and memory as well as neuro-

psychiatric disease, yet the molecular events that control asymmetric brain function and plasticity remain elusive. Here, we show that *Caenorhabditis elegans* utilizes and restructures an asymmetric neural circuit during associative salt-learning. Naïve worms memorize and prefer the salt concentration they experience in the presence of food through a left-biased neural network architecture. We find that animals conditioned at elevated salt concentrations display a change of anatomical connectivity in the salt-learning circuit from a left-biased to a right-biased circuit. The restructured, right-biased circuit responds to decreases in salt concentration with increased turning probability, thus correlating connectivity with salt seeking locomotory behavior of conditioned animals. In contrast, unconditioned animals with a left-biased circuit respond to increases in salt concentration with more turns. This change in circuit architecture requires an asymmetrically expressed, paracrine insulin-like peptide, which signals through the insulin receptor on the primary salt-sensing neurons to asymmetrically add new synapses onto a sensory neuron.Therefore, experience-dependent changes in an animal›s connectome can be mediated by insulin signaling and are fundamental to learning and behavior.

751A **Sexually dimorphic nutrient-dependent behavioral prioritization in *C. elegans*** Chance Bainbridge[1], Gregory Reilly[2], Jinxin Wang[2], Douglas Portman[21]Biomedical Genetics, University of Rochester Medical Center, [2]University of Rochester Medical Center

To cope with nutrient deprivation, animals often reprioritize behaviors to favor feeding over exploring. Because nutritional requirements and reproductive strategies differ by sex, this reprioritization can be sexually dimorphic. Work from our lab and others indicates that *C. elegans* exhibit sexually dimorphic behavioral and neuronal responses to nutrient availability. However, the mechanisms by which biological sex regulates neuronal function to produce sex-specific responses to nutrient status is poorly understood. In *C. elegans*, starvation and re-feeding provides a paradigm to understand how nutritional status and biological sex intersect to modulate the priority of behavioral states.

Here, we investigate sex differences in behavioral priority by profiling distributions of locomotor states in well-fed and previously starved males and hermaphrodites. Because we are interested in roaming, dwelling, and quiescence, we recorded animals on high-quality HB101 food. From these recordings, we trained a Random Forest supervised machine learning model to identify these three states in both sexes. Our results indicate that male worms exhibit nutrient-dependent strategies distinct from hermaphrodites: males maintain high exploration (roaming) and quiesce less than hermaphrodites even following substantial nutrient deprivation. To ask how biological sex may regulate nutrient-dependent locomotor behaviors, we manipulated the genetic sex-determination pathway to sex-reverse specific tissues. These results suggest that the sexual states of both the nervous system and the intestine play a nutrient-dependent role in regulating locomotor state. To determine how nutritional status might be modulated by biological sex, we tested insulin and IGF signaling (IIS) pathway mutants for changes in nutrient-dependent locomotor behavior in both sexes. Preliminary results suggest that increased insulin signaling in males may promote sex-specific nutrient-dependent behavior. These studies will generate a framework to explore potentially conserved mechanisms by which genetic sex regulates neuronal and behavioral responses to nutritional status.

752A **Electrical synapses and the *C. elegans* connectome** Ben Mulcahy[1], Daniel Witvliet[1], Richard Schalek[2], Jeff Lichtman[2], Aravinthan Samuel[2], Mei Zhen[11]Lunenfeld-Tanenbaum Research Institute, [2]Harvard

The Mind of a Worm identified ~7000 chemical synapses and ~600 electrical synapses that make up synaptic connectivity in the *C. elegans* nervous system. Chemical synapses are relatively easy to identify from their ultrastructure, however electrical synapses are much harder to identify. This results in a high degree of subjectivity in electrical synapse annotation, and selective underannotation of smaller synapses. To overcome these difficulties, we developed a protocol to selectively stain electrical synapses in electron micrographs for connectomics. We are using this approach to build a detailed map of high-confidence electrical synapses across the *C. elegans* nervous system and understand how they interact with chemical synaptic connectivity and neuronal morphology in developing nervous systems.

753A **Hierarchical behavior control by a single class of interneurons** Jing Huo, Tianqi Xu, Mahiber Polat, Quan WenUniversity of Science and Technology of China

Animal behavior is organized into nested temporal patterns spanning multiple timescales. This behavior hierarchy is believed to arise from a hierarchical neural architecture: neurons near the top of the hierarchy are involved in planning, selecting, initiating, and maintaining motor programs while those near the bottom of the hierarchy act in concert to produce fine spatiotemporal motor activity. In *Caenorhabditis elegans*, behavior on a long timescale emerges from ordered and flexible transitions between different behavioral states, such as forward movement, reversal, and turn. On a short timescale, different parts of the animal body coordinate fast rhythmic bending sequences to produce directional movements. Here, we show SAA, a class of interneurons that enable cross-communication between dorsal and ventral head motor neurons, play a dual role in shaping behavioral dynamics on different timescales. On the short timescale, SAA regulate and stabilize rhythmic bending activity during forward movements. On the long timescale, the same neurons suppress spontaneous reversals and facilitates reversal termination by

inhibiting RIM, an integrating neuron that helps sustain a behavioral state. These results suggest that feedback from a lower-level cell assembly to a higher-level command center is essential for bridging behavioral dynamics at different levels.

754A **Reverse genetic screen of Parkinson's disease-susceptibility genes identifies novel modulators of alpha-Synuclein neurotoxicity in *C. elegans*** Roman Vozdek, Andrew A Hicks EURAC Research

Neurotoxicity of alpha-synuclein (aSyn) is a pathogenetic hallmark of synucleinopathies, including Parkinson's diseases (PD). Only about 10% of diagnosed Parkinson's disease (PD) have familial history with identified genetic variations, while pathogenetic triggers in sporadic forms of PD are largely unknown. Genome-wide association studies over recent years have revealed approximately 90 risk genetic loci associated with developed PD. To date, however, there is little to no functional validation of genes in these loci. In this study, we performed reverse genetic screening of some of these candidate risk genes, looking for modulated toxicity of aSyn in dopaminergic neurons of *C. elegans*. We generated *C. elegans* PD model expressing GFP-tagged aSyn in dopaminergic neurons, which forms aSyn inclusions and triggers neurodegeneration in aged animals. Using RNA interference, we targeted *C. elegans* orthologs of 100 human risk genes for PD from the published GWAS loci and identified knockdown animals with exacerbated or alleviated aSyn-induced neurodegeneration. We show that several genes regulating calcium signalling modulated aSyn toxicity in dopaminergic neurons and conclude that the genes regulating import of calcium into mitochondria are potential therapeutic targets for PD.

755A **Pheromone-based animal communication influences the production of somatic extracellular vesicles** Agata Szczepanska[1], Katarzyna Banasiak[2], Klaudia Kolodziejska[1], Abdulrahman Ibrahim[3], Wojciech Pokrzywa[2], Michal Turek[11]Laboratory of Animal Molecular Physiology, Institute od Biochemistry and Biophysics of Polish Academy of Sciences, [2]International Institute of Molecular and Cell Biology, [3]Warsaw University of Technology

Extracellular vesicles (EVs) are involved in multiple biological processes. To date, most studies have focused on the intracellular molecular mechanisms of EVs biogenesis and consequently, there is limited knowledge of the influence of environmental factors or other individuals in the population on the activity of EV-regulated systems at the organismal level. We have previously shown that the largest evolutionarily conserved EVs, exophers, are a component of the *C. elegans* maternal somatic tissue resource management system induced by the embryos developing *in utero*. As a result, the progeny of individuals with active exopher biogenesis (exophergenesis) appear to be better adapted to thrive in the habitat. Using this model, we investigated the inter-tissue and social regulatory mechanisms of exophergenesis. We found that exophergenesis activity is differentially modulated by sex-specific ascaroside (pheromones) signaling molecules, known to have multiple functions in development and behavior. While hermaphrodite-released pheromones down-regulate exophergenesis, male-released pheromones favor strong exopher production. This ascaroside-dependent regulation is fine-tuned by exopher-promoting olfactory neurons exposed to the environment (partially via STR-173 seven TM receptor) and exopher-inhibiting sensory neurons exposed to the body cavity. Together, we uncovered critical control nodes for somatic EVs production mediated by the nervous system in response to social cues.

756A **Role of GLR-1 In Age Dependent Memory Decline** Vaibhav Gharat, Dominique J.F. de Quervain, Andreas Papassotiropoulos, Attila Stetak University of Basel

Normal ageing is often accompanied by a deterioration of cognitive functions, including memory decline, which can decrease the functional independence of individuals and increase the risk of Alzheimer's Disease. In a society with a growing elderly population, it is of increased relevance to understand the impact of ageing on cognition, in order to find strategies that can prevent or limit the loss of cognitive functions. Age-dependent memory decline is associated with structural and functional changes in the brain, including alterations in neuronal structure, loss of synapses and decreased plasticity. Studies in vertebrates have previously highlighted the role of dysregulated glutamate receptor signalling in age-related plasticity decline. However, the mechanisms regulating the abundance, distribution and properties of AMPA-type glutamate receptors are still largely unknown. We previously showed in *C. elegans* that the tissue-specific knockout of the AMPA-type glutamate receptor, GLR-1, in the AVA interneurons resulted in impaired olfactory associative memory (Vukojevic et al. 2012). In the current study, we investigated the abundance of total and membrane-bound GLR-1 receptors in the AVA interneurons of wild-type worms and found a significant decrease with age. This decrease in abundance also correlated with physiological age-dependent memory decline. On the contrary, worms lacking *msi-1* (Hadziselimovic et al. 2014) did not exhibit changes in their memory performance with ageing and did not show an age-dependent decline in GLR-1 abundance. Moreover, we studied the dynamics of GLR-1 turnover using FRAP and found that the receptor dynamics reduced significantly with age in wild-type animals as compared to mutants with increased memory performance. Finally, to investigate the link between decreased GLR-1 abundance, decreased receptor dynamics and age-dependent memory decline, we evaluated these processes in transgenic worms expressing a ubiquitination defective variant of GLR-1(4KR). Using the 4KR mutant strain to perform behavioral assays, we showed that restoring GLR-1 abundance in aged animals restores the GLR-1 dynamics and ultimately improves cognitive performance.

757A ***Caenorhabditis elegans* model of Riboflavin Transport Deficiency (RTD) disorder shows reduced growth, synaptic transmission defects and locomotion deficits** Ramesh Narayanan[1,2], Brent Neumann[3], Manoj Menezes[4], Marina Kennerson[1,2,51]ANZAC Research Institute, [2]Sydney Medical School, University of Sydney, [3]Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, [4]5NTY Nelson Department of Neurology and Neurosurgery, Children's Hospital at Westmead, [5]Molecular Medicine Laboratory, Concord General Repatriation Hospital

Riboflavin Transport Deficiency (RTD) is a rare neurodegenerative disorder caused by mutations in the solute carrier family 52 member 2 (*SLC52A2)* gene. Riboflavin transporter genes *rft-1* and *rft-2* are the worm orthologs for human *SLC52A2*. RFT-1 is predominantly expressed in neurons while RFT-2 expression is restricted to the intestine in worms. The *rft-1* gene was chosen for knocking in RTD mutations due to the spatio-temporal expression in neurons, the cell type affected in RTD. Homozygous knock-in mutants were generated using CRIPSR-Cas9. Animals carrying *rft-1*^{Y290C} corresponding to the human *SLC52A2*^{Y305C} mutation and the *rft-1*^{L324P} corresponding to the human *SLC52A2*^{L339P} mutation were generated. *rft-1*^{L324P} animals were not viable due to embryonic lethality. Results using viable *rft-1*^{Y290C} animals are discussed in this study. Biochemical analysis using aldicarb (a helminthicide) is widely used to screen for synaptic transmission mutants in *C. elegans*. *rft-1*^{Y290C} animals were found to be hypersensitive to aldicarb indicating that knock in of the RTD mutation leads to synaptic transmission defects. In addition to displaying reduced body width, *rft-1*^{Y290C} animals showed a slight reduction in thrash count suggesting growth and locomotion defects respectively. However, the neuron morphology of GABAergic motor neurons showed no signs of neurodegeneration. CeNGEN data suggested that RFT-1 is highly expressed in the AVL (motor) neuron responsible for defecation. *rft-1*^{Y290C} animals displayed a reduced defecation rate suggesting AVL motor neuron dysfunction. Recent work demonstrated that knockdown of *rft-1* led to reduced bioenergetics and increased fat storage in *C. elegans*. ATP production and mitochondrial content were normal in our RTD *C. elegans* model. Although not statistically significant, *rft-1*^{Y290C} animals displayed a trend of increased fat storage when compared to control animals. In summary, the RTD mutants generated in this study clearly demonstrate growth defect indicative of metabolic dysregulation and abnormal synaptic transmission. This suggests nervous system dysfunction could be used as a phenotypic read-out in future screening studies using our model to identify drugs that can ameliorate nervous system related problems in RTD patients.

758A **Multi Time-scale Neuronal Activities in the Brains of Freely Moving C. elegans** Charles Fieseler, Ulises Rey, Lukas Hille, Itamar Lev, Manuel Zimmer Neuroscience and Developmental Biology, University of Vienna

Recent neuroscience has seen various paradigm shifts due to the availability of large-scale neuronal activity datasets obtained by multi-electrode arrays or Ca2+-imaging techniques. In the nematode C. elegans it has recently become possible to image activity of the entire brain at single-cell resolution in freely moving animals. We have established a) an imaging pipeline based on a speed-optimized spinning disk microscope that enables worms to crawl normally and b) a computational pipeline that allows automated analysis and manual correction of neuron segmentation and tracking. Previous analysis in immobilized worms has shown population-wide activity described by a low dimensional manifold formed by clusters of neurons functionally associated with behavioral states like the backward- and forward- crawling motor programs. While state dependent locking for many of these neurons has been confirmed on an individual neuron basis in freely moving animals, it was unclear how their population activity appears under unrestrained conditions. Consistent with the previous findings, we observe population wide activity in the form of a structured manifold in freely moving animals which has striking similarities to the one found in immobilized animals, capturing the forward-backward behavioral transition. In addition, previous work has hypothesized and partially shown that there should be more complex neuronal representations related to ongoing behaviors that operate on faster time-scales than switching between forward-backward states, like subtle head motions and body undulations. We show that at faster timescales there are oscillations that correlate with instantaneous body curvature. In summary, we show in freely crawling animals that information about the current slow-time scale behavioral states is shared across a large population of neurons, while fast action motifs are represented more locally by individual neuronal classes. We suggest this as an organizational principle that could apply to animals with larger brains.

759A **Development of a machine learning classifier to predict GPCR-peptide interactions** Larissa Ferguson[1], Isabel Beets[2], William R Schafer[11]Neurobiology Division, MRC Laboratory of Molecular Biology, [2]Department of Biology, KU Leuven

Neuropeptide-GPCR signalling networks play a crucial role in the nervous system of C. elegans, and the importance of these networks likely extends to other species. However, our ability to map such networks is limited by our knowledge of GPCR-peptide interactions in a given species, and we currently lack the ability to identify potential interacting pairs without extensive experimentation. To address this, we have developed a machine learning model to predict GPCR-peptide interaction based on primary sequences. We trained a random forest classifier to distinguish interacting and non-interacting GPCR-peptide pairs from primary sequence using a dataset of experimentally verified interactions in C. elegans (Beets et al., 2022, bioRxiv). We utilize protein representations generated by deep learning algorithms for predicting protein structure, such as ESMFold and AlphaFold2, and assess their suitability for predicting GPCR-peptide interactions. To address the class distribution imbalance inherent in the

dataset, we employed stratified k-fold cross-validation during training and hyperparameter tuning. While the model has high predictive success within C. elegans, we aim to optimize the model for cross-species predictions by incorporating cross-species data in the training, dataset partitioning based on promiscuity and sequence similarity, and feature transformation to reduce dimensionality of deep learning embeddings used to represent the proteins. Ongoing work aims to identify and modify protein representation features contributing to predicted interaction to generate novel peptide ligands with specific GPCR binding profiles in C. elegans. To assess the reliability and generalizability of our model, we plan to experimentally test predicted novel GPCR-peptide interactions using electrophysiology in addition to the calcium mobilization assay utilized in acquisition of the original dataset. This project showcases the practicality of using deep learning-based protein representations for predicting protein-protein interactions, and offers a valuable resource for broader explorations into GPCR-peptide interactions in C. elegans.

760A **Long-term posture dynamics across development reveals stereotyped and individual-specific behavioral signatures** Yuval Harel[1], Shay Stern[21]Faculty of Biology, Technion - Israel Institute of Technology, [2]Technion - Israel Institute of Technology

Patterns of behavior across development are formed at multiple timescales, from typical movements over seconds and minutes to patterns of behavior that span the lifetime of the organism. The basic building blocks that characterize the animal's movement are changes in its postures. The available modes of movements can then be integrated to form a complex pattern of behavior that reflects a composition of much simpler movements. Posture dynamics during adulthood were shown to form stereotypic movements that can be studied both at the behavioral and neuronal level. However, how the spectrum of underlying posture modes changes across all developmental windows and what are the variations in posture modes among individuals is not known.

Here, we study *C. elegans* behavior and individuality using long-term monitoring of posture dynamics of individual worms in a controlled environment, from hatching to adulthood, across their complete development time. Our database includes over 1000 individuals imaged at 3fps, from which we extracted ~0.5 billion body postures by analyzing each frame. We used an unsupervised analysis to identify the typical repertoire of posture dynamics on a short time scale, as well as individual variation in posture dynamics on a developmental time scale. Specifically, we perform dimensionality reduction of posture dynamics and use it to characterize the long-term patterns of each individual within these low-dimensional representations.

These analyses reveals dominant modes of posture dynamics and how they change across development, as well as temporal patterns of individuality within the population. Comparison across different genotypes reveals how specific neuromodulators affect these stereotyped and individual-specific temporal patterns.

761A **Neural activity-independent regulation of synaptic vesicles by K2P channels.** Jun Meng[1,2], Neeraja Ramakrishnan[1], Yi Li[3], Mei Zhen[11]Lunenfeld-Tanenbaum Research Institute, [2]Katholieke Universiteit Leuven, [3]Huazhong University of Science and Technology

One of the key questions in neural developmental biology is how synapses develop and maintain their function. Two-pore potassium (K2P) channels are broadly expressed and function in all animals and are originally thought to conduct background potassium leak current and maintain the resting membrane potential (RMP) of cells, hence cell excitability. Recent cumulative evidence shows the diverse roles of K2P in many essential biological signaling and processes in addition to regulating RMP. However, the physiological relevance of K2P's function in synapse development and neural circuit function remains unknown. Through genetic screens, I discovered that mutations in *twk-2* and *twk-40*, *C. elegans* K2P encoding genes, affect synaptic vesicle protein expression and neural functions likely in a activity-independent maner. Here, I will present and discuss the molecular mechanisms underlying K2P›s regulation of synapse vesicles. My research suggests a superising mechanistic role of K2P in neural development and circuit function.

762A **Intentionally left blank**

763A **m6A DNA methylation controls forgetting of long-term memories in *C. elegans*** Lea Kaspar[1], Andreas Papassotiropoulos[2], Attila Stetak[21]Max-Planck Institute of Psychiatry, [2]Molecular Neurosciences, University of Basel

Understanding neural circuits and molecular mechanisms underlying changes of synapse strength during learning and memory are the major challenges of neuroscience. While the mechanisms of learning and memory are widely studied, the decay of memories (forgetting), a poorly investigated but apparently highly complex mechanism, is also essential for proper functioning of the brain.

Recently, we identified a core set of genes differentially regulated during long-term memory, out of them *nmad-1* represents the *C. elegans* homolog of DNA m6A demethylase enzyme (Freytag et al., 2017). Using an aversive olfactory associative long-term memory test, we confirmed that *nmad-1* gene is indeed transcriptionally upregulated; levels peaking 4h post-training.

Furthermore, *nmad-1* loss-of-function mutants surprisingly have an increased long-term but normal short-term memory performance compared to wild-type worms. We generated a knock-out mutant of the DNA adenosine methyltransferase gene, *damt-1*, likely acting opposing to the *nmad-1* function. As expected, deletion of the *damt-1*impaired long- but not short-term memory suggesting that dynamic modification of the DNA m6A modification plays and important role in long-term memory maintenance and elimination. Using tissue specific rescue experiments we demonstrated that *damt-1* function is required in AVA neuron. Additionally, immunostaining with m6A-specific antibody showed reduced DNA 6-adenosine methylation specifically in AVA neuron upon long-term memory training, while global or AVA-specific RNA methylation levels were not affected. Finally, we compared gene expression differences in wild-type, *nmad-1* and *damt-1* mutant worms and compared global gene expression profiles and also filtered with the previously defined core memory regulated gene-set in order to identify the methylation regulated memory genes.

Altogether, we show that DNA m6-adenosine methylation plays a role in associative long-term memory in worms. Since *nmad-1* expression level increases during memory and deletion of the gene results in enhanced memory performance, this may represent a controlled forgetting mechanism in the AVA neuron in *C. elegans*. As DNA m6A methylation was recently described in humans as well, our findings likely represent an evolutionary conserved forgetting mechanism also present in vertebrates.

764A **Quantitative Behavioural Phenomics: *C. elegans* as a model organism for the development of high-throughput precision medicine** Thomas J O›Brien[1,2], Andre EX Brown[1,21]Institute of Clinical Sciences, Imperial College London, [2]MRC London Institute of Medical Sciences

The goal of precision medicine is to tailor treatments for individuals based upon genomic sequencing data. However, many diseases are associated with mutations in multiple genes, and drugs often interact with multiple targets, making the link between genetic diagnosis and drug treatment challenging. This is particularly true for rare disorders, where the identification of a genetic lesion may not lead to a candidate target because the cause is a complete loss of function of the causal gene and/or because there is little understanding of the genetic pathways that are implicated. Hence, phenotypic screens in model organisms provide a tractable route to identify novel drug treatments. However, lack of readily-screenable phenotypes still remains a barrier in the development of efficient high-throughput screening campaigns to identify treatments for rare genetic disorders. We are using *Caenorhabditis elegans* as a model organism to redress this issue and investigate fundamental questions about the mapping between chemical/genetic perturbation and behavioural phenotypes.

We have developed high-throughput, automated tracking technologies capable of measuring changes in behaviour as the result of genetic mutation, or treatment with small molecules/metabolites. To test the power of our approach in treating human genetic disorders, we have generated a panel of *C. elegans* strains containing mutations in genes orthologous to those associated with rare human neuromuscular disorders. As such, these mutant worm strains are considered patient-specific 'avatars' of individual rare genetic diseases. Despite some mutations leading to incongruous phenotypes imperceptible to the naked eye, they can (in most cases) be captured by quantitative phenomics. The resulting 'behavioural fingerprints' provide a high-content view of multidimensional phenomic space for individual genetic disorders. We have successfully used these to perform large compound library screens with high-throughput, and identify novel drug repurposing candidates with the potential to treat rare (often poorly characterised) genetic disorders with no prior knowledge of the molecular basis of disease beyond the patient mutation. Ultimately, this work will enable the development of a high-throughput precision medicine platform to identify candidate compounds for the treatment of many genetic disorders, using an individual's personalised *C. elegans* disease-model avatar.

765A **Functions of anti-microbial peptides in neural circuits and behaviour** Xinyi RESEARCH YANG[1], Nathan de Fruyt[2], Isabel Beets[3], William RESEARCH Schafer[41]MRC Laboratory of Molecular Biology, [2]Department of Biology KU Leuven, [3]Department of Biology, KU Leuven, [4]Department of Neurobiology, MRC Laboratory of Molecular Biology

Infections caused by pathogenic microbiota are believed to alter the behaviours of animals, yet the mechanisms underlying this are not well understood. Recent research in immunology and neuroscience has shown that the activation of immune cells can stimulate neuron circuits, which will in turn regulate both innate and adaptive immune response. However, it remains unclear how small, infection-related neuropeptides could contribute to the neuro-immune interactions.

C. elegans contains several structurally-related antimicrobial peptides (AMPs) that are induced under pathogenic conditions. We observed that two of these AMPs, *nlp-29* and *nlp-24*, affect *C. elegans* behaviour in specific ways. *nlp-29* knock-out worms survive longer on lawns of the pathogen *S. marcescens* compared to wild type worms, an enhancement we observed to be coupled with altered avoidance behaviour from pathogenic bacterial. Specifically, whereas wild-type worms avoid pathogenic bacteria following exposure and infection, *nlp-29* mutants remain attracted to pathogenic bacteria and remain in lawns of Serrati. This altered avoidance behaviour of mutants can be rescued by expressing *nlp-29* in amphid and phasmid sheath cell glia promoter, suggesting that APMs released from glia modulate olfactory navigation circuits following pathogen exposure.

The G protein-coupled receptor NPR-12 has been reported to act as the receptor of NLP-29 peptide. We observed similar lifespan and avoidance behaviour phenotypes in *npr-12* mutants as in *nlp-29* mutants. We also observed that NLP-29 peptides can activate NPR-12 expressed in *Xenopus* oocytes, where it appears to signal through the $G_{i/o}$ pathway. We are currently carrying out rescue experiments to identify the neurons in which NPR-12 functions to modulate pathogen avoidance. We also aim at discovering the down-stream signalling pathways of this anti-pathogen behaviour modulation and the possible immunity pathways that trigger NLP-29 function during infection.

We also observed that a different AMP, NLP-24, affects salt gustatory plasticity, a simple form of associative learning. Specifically, *nlp-24* knock-out worms reduced gustatory plasticity as compared to wildtype, implying that AMPs could affect worm behaviours unrelated to pathogenic conditions. Experiments in *Xenopus* oocyte showed that NLP-24 peptide can activate NPR-17, indicating that NPR-17 may act as the receptor of NLP-24 peptide for its learning functions. We are now focusing on assessing the learning phenotype of *npr-17* mutants and specifying the neurons or other cells where NLP-24 modulates the learning behaviour.

766A **Visualising neuromodulation *in vivo*** Emma Clark[1], Evie Goss-Sampson[2], Ángela Jimeno Martín[2], Jan Watteyne[3], Isabel Beets[3], Arantza Barrios[11]Cell and Developmental Biology, University College London, [2]University College London, [3]Department of Biology, Katholieke Universiteit Leuven

In order to survive, animals must adapt their behaviour to withstand changes in their environment. Such behavioural flexibility arises from alterations within neural circuits, mediated by molecules known as neuromodulators. A major class of neuromodulators are neuropeptides; small, diffusible molecules which can act extrasynaptically to alter the dynamics and composition of neural circuits. In this way neuropeptides have the potential to act on all receptor-expressing targets, yet they regulate behaviour in a specific manner by acting on distinct sets of neurons in different contexts. What mechanisms determine a neuropeptide's spatial range of action?

To answer this question and thus understand how neuropeptide transmission achieves circuit specificity, we are adapting a system to visualise neuropeptide receptor activation in *C. elegans*. We are implementing the TANGO assay, which converts transient ligand-receptor interactions into stable expression of fluorescent transgenes, to map out the neurons that receive neuropeptide signals. Specifically, we are using Pigment Dispersing Factor (PDF) and its receptor (PDFR-1) as a model system, as PDF signalling mediates different behaviours depending on the source of release and context.

We will utilise the *C. elegans* TANGO strain to identify mechanisms that facilitate or constrain neuropeptide diffusion and spatial range of action. As many cognitive and mood-related disorders are caused by dysfunctional neuropeptide signalling, it is vital to understand how information is coded by neuromodulation.

767A **CaMKII mediates sexually dimorphic synaptic transmission at neuromuscular junctions in *C. elegans*** Wan-Xin Zeng[1], Haowen Liu[2], Yue Hao[1], Kang-Ying Qian[1], Fu-Min Tian[1], Lei Li[2], Bin Yu[3], Xian-Ting Zeng[1], Shangbang Gao[3], Zhitao Hu[2], Xiajing Tong[11]ShanghaiTech University, [2]Queensland Brain Institute, [3]Huazhong University of Science and Technology

Sexually dimorphic behaviors are ubiquitous throughout the animal kingdom. Although both sex-specific and sex-shared neurons have been functionally implicated in these diverse behaviors, less is known about the roles of sex-shared neurons. Here, we discovered sexually dimorphic cholinergic synaptic transmission in *C. elegans* occurring at neuromuscular junctions (NMJs), with males exhibiting increased release frequencies, which causes sexually dimorphic locomotion behaviors. Scanning electron microscopy revealed that males have significantly more synaptic vesicles (SVs) at their cholinergic synapses than hermaphrodites. Transcriptome analysis identified the male-enriched transcripts and focused our attention on CaMKII/UNC-43. We ultimately show that differential UNC-43 accumulation at cholinergic neurons controls axonal SV abundance and synaptic transmission. Finally, we demonstrate that the sex-reversal of pan neurons in hermaphrodites is able to generate male-like cholinergic transmission and locomotion behaviors. Thus, beyond demonstrating CaMKII/UNC-43 as an essential mediator of sex-specific synaptic transmission, our study provides insights at the molecular and cellular levels about how sex-shared neurons can generate sexually dimorphic locomotion behaviors.

768A **NeuroPAL: Whole-Nervous-System Cell IDs to Map Neural-Communication and Cell-Fate Dynamics in Live Animals** Xingyang Fu[1], Kevin W Rusch[1], Maedeh Seyedolmohadesin[2], Mahdi Torkashvand[2], Vivek Venkatachalam[2], Eviatar Yemini-[11]Neurobiology, UMass Chan Medical School, [2]Department of Physics, Northeastern University

A major challenge in biological imaging is resolving cell identities. These are necessary to determine individual neurons' contributions to brainwide activity and behavior, cell-fate dynamics (e.g., when disrupted by mutations), and cell-specific protein expression and function.

We recently published a novel method and software that resolves the cell-type identity of every neuron *in vivo*, across developmental stages, and inclusive of both sexes, using a single animal strain we call "NeuroPAL" (a **Neuro**nal **P**olychromatic **A**tlas of **L**andmarks) [1,2]. Here we showcase a variety of applications and results in *C. elegans*, highlighting our new findings and updates to the NeuroPAL method.

Using NeuroPAL, we identified brainwide codes for a panel of attractive and aversive stimuli, revealing their high-dimensional complexity. We then compared coding between both sexes and discovered extensive differences between their neuron-specific and circuit-level stimulus responses. By mapping all classical metabotropic neurotransmitter signaling across the entire nervous system, we uncovered a broadcast network of inhibitory control and extensive potential for extrasynaptic GABA signaling. Lastly, we used NeuroPAL to investigate cell-fate dynamics during development, thereby determining the precise timing of neural differentiation and dissecting the roles of various conserved cell-fate regulators (e.g., miRNA's, bHLH's, and other transcription factors) in controlling neural identity, cell duplication or absence, and positional displacement [1,2,3].

Together, these NeuroPAL applications demonstrate the power and potential of our unique system for addressing previously difficult and unanswerable questions in neuroscience. In our continuing commitment to help other labs investigate nervous-system communication and architecture, we provide multiple updates to the NeuroPAL ID software and manuals that guide and automate the workflow. NeuroPAL strains, plasmids, manuals, software, code, and datasets are widely available from the Caenorhabditis Genetics Center (CGC), Addgene, and our website www.yeminilab.org/neuropal.

[1] Yemini, E *et al*. Cell (2021).

[2] Tekieli, T, *et al*. Development (2021).

[3] Masoudi, N *et al*. Development (2021).

769A **The central role of the transcription factor CREB in the behavioral changes induced by starvation and glucose exposure** Laura Gabriela Gutierrez, Mariana Zurita, Francisco Pinta, Nallely Cano, Julian Valdes UNAM IFC

C. elegans has a remarkable capacity to sense the environment and adapt its behavior, even by establishing long-term memory of the ecosystem. In this sense, the nutritional state of the animal influences the metabolism affecting foraging and escape behaviors. We found that transient periods of starvation during larval stages or adults result in changes in the preference for odorants. These alterations depend on sRNA metabolism, the insulin pathway, epigenetic regulators and CREB, a transcription factor involved in histone acetylation and gene activation. CREB activity was detected in neurons and intestine cells during starvation but remained only in the intestine after re-feeding of the animals. Tissue-specific silencing of CREB showed that is essential in both tissues, although H3K27 acetylation differences were not detected after starvation. On the other hand, exposing the nematodes to a high-glucose environment not only affects the worm›s preferences for odorants but also impairs the establishment of a long-term memory to the odorant benzaldehyde. Both, the insulin pathway and CREB are essential for glucose-induced behavioral changes, but CREB activity was detected only in the intestine cells under glucose exposure. Transcriptional profiling of the worms showed that both insults resulted in alterations in metabolic pathways. Our results highlight the central role of CREB as a master regulator of stress responses in the worm and in the crosstalk between the intestine and the nervous system when integrating environmental clues with behavioral changes.

770A **Specific glial regulators of ions and solutes are required for different chemosensory function in *C. elegans*** Lei Wang, Bianca Graziano, Nicole Encalada, Jesus Fernandez-Abascal, Daryn H Kaplan, Laura Bianchi Physiology and Biophysics, University of Miami

Anosmia is among the most prevalent symptom of SARS-CoV-2 infection. Interestingly though, the SARS-CoV-2 virus infects the olfactory supporting cells, rather than the primary sensory neurons themselves. These recent findings have underscored the importance of the supporting cells in olfaction. However, very little is known about the mechanisms underlying the regulation of olfaction by the supporting cells. In *C. elegans*, the Amphid sheath (Amsh) glial cells are the supportive cells of the amphid sensory apparatus, sharing with mammalian olfactory supporting cells general function and expression of the homologs of the SARS-CoV-2 viral entry proteins ACE2 and TMPRSS2. To understand the contribution of the Amsh glia to sensory function, our lab has taken the unbiased approach of sequencing the mRNA extracted from these cells. Among the ~1,000 glial-enriched genes, we identified 14 ion channel and transporter genes with 2.7- to 29.6-fold mRNA enrichment in Amsh glia as compared to other cells. To determine whether these channel and transporter genes are needed for sensory behaviors, we performed behavioral assays on knock-outs and Amsh cell specific knock-downs. Our results support the predominant requirement of glial K^+ and Cl^- channels and transporters for the response to isoamyl alcohol, octanol, diacetyl, and Na-acetate. Ca^{2+} imaging experiments in Amsh glia and neurons further support the requirement of these channels in glia for the function of glia and associated neurons. Taken together, our findings expand on our understanding of the mechanisms underlying the

contribution of Amsh glia to sensory perception in *C. elegans*; mechanisms that might be conserved from worm to man.

771A **Genetic analysis of circuit connectivity identifies key processes important for the development and maturation of excitatory synaptic connections to GABAergic neurons** Michele L Lemons[1], Devyn B Oliver[2], Kasturi Biswas[2], Julia Russell[2], Hailey McKillop[1], Michael M Francis[21]Biological and Physical Sciences, Assumption University, [2]Neurobiology, University of Massachusetts Chan Medical School

A high degree of cell and circuit-specific regulation has presented challenges for efforts to precisely define molecular mechanisms controlling synapse maturation and circuit connectivity. Here, we pursue an unbiased forward genetic approach to identify *C. elegans* genes involved in the formation and maturation of cholinergic synaptic connections with GABAergic motor neurons, as indicated by alterations in the distribution of GFP-tagged acetylcholine receptors (AChRs) in GABAergic dendrites. We identified mutations in three genes that are important for three different key processes in synapse/circuit maturation: postsynaptic receptor assembly, trafficking of synaptic cargoes, and synapse structural organization. We found that mutation of the nicotinic acetylcholine alpha subunit *unc-63* caused a failure in AChR assembly in GABAergic neurons but did not significantly alter dendritic spine structure or abundance. In contrast, mutation of the RUN domain (RPIP8, UNC-14, and NESCA) cargo adaptor *unc-14* dramatically impacted both dendritic spines and overall GABAergic neuron morphology. Notably, specific expression of wild type *unc-14* cDNA in either GABAergic neurons or presynaptic cholinergic neurons was not sufficient to rescue the *unc-14* mutant phenotype while pan-neuronal expression provided significant rescue, indicating that disruptions in GABAergic neuron morphology arise from compound effects. Finally, we obtained a mutation in the Liprin-α synaptic scaffold *syd-2* that produces a stop codon in a C-terminal SAM domain and has severe effects on dendritic spines and AChR localization. Our unbiased strategy identified key genes that implicate three distinct cellular processes important for synapse circuit development and maturation, and highlight how mechanisms for receptor assembly, cargo trafficking and synapse structural organization each contribute to circuit connectivity.

772A **Innexin hemichannel regulates transmission of temperature information during *C. elegans* thermotaxis** Airi Nakayama[1], Riku Yamashiro[1], Hiroo Kuroyanagi[1], Ikue Mori[1], Shunji Nakano[2,21]Nagoya University, [2]Department of Biological Science, Nagoya University

Innexins and connexins are components of hemichannels, which, through docking with other hemichannels from adjacent cells, form gap junctions. While gap junctions regulate electrical synaptic transmission, recent studies on mammalian astrocytic connexin implied that hemichannels could coordinate neural communication. Here we report that neuronal innexin hemichannel regulates the transmission of temperature information during *C. elegans* thermotaxis. Encouraged by our previous finding that INX-4, a member of innexin family, is required for thermotaxis (Tsukamoto et al. 2020), we assessed whether other innexin genes are involved in the regulation of thermotaxis. Our genome-wide survey of innexin genes revealed that UNC-7/Innexin is also required for thermotaxis: loss or gain of *unc-7* activity specifically in AFD thermosensory neuron caused thermotaxis defects. 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. These results suggest that UNC-7 acts in AFD and controls transmission of temperature information. To address whether UNC-7 acts as gap junction channels or hemichannels, 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 *unc-7(AFD KO)* mutation in which *unc-7* is knocked out specifically in AFD, suggesting that UNC-7 acts as a hemichannel to regulate thermotaxis. To identify the neural pathway in which UNC-7 functions to regulate thermotaxis, we conducted behavioral component analysis and found that UNC-7 transmits temperature information from AFD to AIY interneuron to regulate curving bias during forward runs. 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 *unc-7(AFD KO)* affected thermotaxis in strains lacking *eat-4* or *unc-31*, suggesting that UNC-7 acts in parallel to chemical synaptic transmissions. Our results suggest that UNC-7 hemichannel acts in neurons and regulates the transmission of temperature information by a mechanism distinct from the known synaptic transmissions.

773A **Social contexts modulate *C. elegans* thermotaxis behavior** Shunji Nakano[1,1], Hiroo Kuroyanagi[2], Riku Yamashiro[2], Airi Nakayama[2], Hironori J Matsuyama[2], Ikue Mori[21]Department of Biological Science, Nagoya University, [2]Nagoya University

The optimal behavioral strategy varies depending on the presence or absence of the competing individuals. While animals can maximize their fitness and reproduction by exploiting the available resources in the absence of the competitors, they would have a better chance for survival by exploring a new environment when surrounded by the competing individuals. However, the molecular and neural mechanisms underlying such a decision-making remain elusive. We recently discovered that the *C. elegans* nervous system can perform such a neural computation: animals alter thermotaxis behavior, depending on the population density in the environment. While the animals cultivated under a low population density exploit their previous

experience and prefer the temperature at which they have been previously cultivated with food, they start exploring a new temperature when surrounded by a large number of the conspecifics. We identified from a genetic screen that *rdl-1*, which encodes a homolog of the mammalian Retinal Degeneration 3 protein known to regulate the trafficking of guanylate cyclases, is required for the modulation of thermotaxis. *rdl-1* mutants abnormally explored temperatures other than the cultivation temperature even under the population density at which the wild-type animals would prefer the cultivation temperature. *rdl-1* acts in four chemosensory neurons -ADF, ASI, ASJ and ASK- sensing the population density and controls the subcellular localization of DAF-11, the guanylate cyclase previously shown to act in the pheromone sensing. These results suggest that the pheromone signaling modulates thermotaxis behavior.
To understand the neural mechanism underlying this behavioral modulation, we conducted cell ablation experiments. Since each of the four chemosensory neurons forms a gap junction with the AIA interneuron, we asked whether AIA is involved in this modulation. We observed that the ablation of AIA suppressed the thermotaxis defect of *rdl-1* mutants. The wild-type animals lacking AIA also failed to alter the thermotaxis behavior under a high population density. Furthermore, *inx-1*, which encodes a gap junction protein, is required in AIA for exploration under a high population density. Our results indicate that social contexts modulate a *C. elegans* decision-making and suggest that gap junctions define a brain state by altering the circuit operation of the sensory information, thereby generating a behavior appropriate for the social context.

774A **Combining human and *in silico* judgement in the search for genetic regulators of nictation** Patrick D. McClanahan[1], Tuan Anh Le[1], Luca Golinelli[1], Bram Cockx[1], Rose Boelen[1], Heeseung Yang[2], Junho Lee[2], Liesbet Temmerman[11]KU Leuven, [2]Seoul National University

Nictation is a dispersal behavior uniquely displayed by dauer life stages of nematodes. Nictating worms stand upright on their tails, often waving back and forth, hypothesized to assist in sensing and/or attaching to another organism. This is relevant in the context of host finding in parasitic nematodes, or as a phoretic behavior of *C. elegans*.

Despite Herculean efforts in past years, only a handful of genes have been found to affect nictation. Details on how they achieve this have not yet been uncovered. This is in part because studying nictation is challenging: the behavior is three-dimensional and can only be observed on textured surfaces; quantification of nictation relies on entire populations of genetically identical individuals; it can only be observed in dauers, and even in isogenic populations, the behavior is hugely variable. Up until now, this meant nictation had to be scored by a human observer in a time-consuming manner prone to unwanted variation.

To achieve a more robust analysis of nictation behavior, we developed a semi-automated pipeline that makes use of computer vision and machine learning to calculate nictation ratio based on videos of recorded dauer behavior. Briefly: Mask R-CNN reliably detects and segments dauers on a textured background, then, quantitative features useful for detecting nictation are computed based on the worms' postural information. Using these features and a human-scored subset of our data, we trained a machine learning classifier to recognize nictation with accuracy similar to that of other human scorers.

Utilizing our pipeline, we show how nictation behavior of *C. elegans* increases as the animals become dauers, clearly correlating with their SDS resistance, and then remains relatively constant for weeks. Our semi-automated workflow further proved useful to score differences in mutant nictation and in nictation behavior depending on growth conditions (liquid *vs.* plate), to observe dauers of different nematode species, and to probe for responses to presented volatile cues.

Because only a few minutes of video data are needed to robustly and longitudinally score behavior of many animals in parallel, our pipeline will be useful to speed up a more robust understanding of nictation, which in turn is expected to increase knowledge on the genetics, ecology, and biology of dispersal, as well as on host finding in economically relevant nematodes.

775A **Lipid peroxidation promotes regenerative axonal fusion and functional recovery after nerve injury** Su-Hyuk Ko, Zhijie Liu, Lizhen Chen University of Texas Health Science Center San Antonio

Functional recovery after nerve injury requires re-establishment of the lost connections. Axonal fusion, a process in which the regrowing axon connects and fuses with its distal axon fragment, represents an efficient to recover function after nerve injury. However, how axonal fusion is induced and regulated remains largely unknown. We discover that ferroptosis inducing signals can promote axonal fusion and functional recovery after axon injury in *C. elegans*. The fusion-promoting effect is dose-sensitive, with a moderate level of ferroptosis signaling leading to axonal fusion, while an excessive level creating axonal debris. Mechanistically, ferroptosis signaling-induced lipid peroxidation enhances injury-triggered phosphatidylserine (PS) exposure to promote axonal fusion through PS receptor (PSR-1) and EFF-1 fusogen. Extending these findings to mammalian nerve repair, we show that loss of GPx4 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

novel insights for developing therapeutic strategies to treat nerve injury.

776A **Selected mRNA decay triggers axon degeneration in adult *C. elegans*** Dong Yan MGM/Cell Biology/Neurobiology, Duke University

Axon degeneration happens during aging and is a hallmark of many neurodegenerative diseases. In the last decade, studies in different model organisms have uncovers some key factors involved in axon degeneration, including NMAT, SARM1/TIR-1, and MYCP-2/RPM-1. However, there are still much to learn about the molecular mechanisms underlying axon degeneration. Using *C. elegans* DD motor neurons as a model, we showed that all known factors involved in axon degeneration are also important for axon degeneration in adult *C. elegans*, supporting that axon degeneration is a conserved biological process from *C. elegans* to mammals. Based on this observation, we designed and carried out an unbiased forward genetic screen and isolated mutants with defects in injury-induced axon degeneration. From analyses of these mutants, we uncovered the critical role of selected mRNA decay in initiating axon degeneration. Furthermore, we confirmed the same molecular mechanism is used in mammalian axon degeneration. Our study reveal novel regulators of axon degeneration and show that *C. elegans* is a powerful model system to study axon degeneration. In this meeting, I will present our unpublished results on how selected mRNA decay triggers axon degeneration in *C. elegans*.

777A **Reciprocal learning enhancement between two conditioned stimuli during classical conditioning in the complex environment** Huijuan Zhao, He Liu Beijing Normal University

The interaction between conditioned stimulus and unconditioned stimulus has been widely studied. However, in the complex environment, several natural cues can be used as conditional stimuli. When one unconditioned stimulus is associated with several natural cues, whether animal form several memories related to different conditioned stimuli, whether there is interaction between conditioned stimuli during classical conditioning in the complex environment. The answers for these questions are largely unclear. Here, we use starvation induced olfactory aversive memory in C. elegans to address these questions. We presented two chemical odors isoamyl alcohol (IAA) and diacetyl when starving the worms and then tested the preference towards individual odors, we found that worms form aversive memories for both isoamyl alcohol and diacetyl, interestingly, the aversive learnings of IAA and diacetyl are enhanced when there are two odor stimuli, comparing with one odor cue as conditioned stimulus during training. Because IAA and diacetyl are sensed by different sensory neurons, next we examined the interaction between IAA and benzaldehyde, both of which are senses by the same sensory neuron AWC, when associated with starvation. We found that benzaldehyde improved the aversive learning of IAA. To further test the learning enhancement of two conditioned stimuli are general in different modalities, we presented the olfactory stimulus IAA and gustatory stimulus no NaCl, interestingly, the gustatory stimulus no NaCl also increases IAA learning. Next, We investigated the neuronal mechanisms for the reciprocal learning enhancement between IAA and diacetyl, we found both interneuron AIY and AIA play important roles for diacetyl enhancing the aversive learning of IAA, however, AIY, but no AIA, is involved in IAA improving diacetyl learning. In this study, we demonstrate a new interaction rule between two conditioned stimuli, it not only deepens the knowledge of the classical conditioning, but also shed light on the method of learning enhancement.

778A **Mind of a dauer: Comparative connectomics reveals developmental plasticity** Junho Lee[1], Hyunsoo Yim[1], Daniel T Choe[1], Alexander J Bae[1], Hae-Mook Kang[1], Ken C.Q. Nguyen[2], Myung-kyu Choi[1], Soungyub Ahn[1], Sang-kyu Bahn[3], Heeseung Yang[1], David H. Hall[2], Jinseop Kim[41]Seoul National University, [2]Albert Einstein College of Medicine, [3]Korea Brain Research Institute, [4]Sungkyunkwan University

The dauer, an alternative developmental stage of nematodes under harsh conditions, is presumed to have a reversibly remodeled nervous system to meet various dauer-specific needs. However, little is known about how extensively the remodeling occurs in dauers. Here, we obtained and analyzed serial-section transmission electron microscopy (ssTEM) volumes of a dauer nerve ring. The volumetric reconstruction revealed dauer-specific structural changes in neural processes, accompanied by local synaptic rewiring among well-preserved neurons. Automated synapse detection by deep learning was adopted to map the complete chemical connectome, which enabled us to compare the dauer connectome with non-dauer ones. While the overall architecture of the nervous system was well preserved, there were dauer-specific connections quantitatively and qualitatively different from those in other developmental stages. We found that those dauer-specific connections are important for dauer-specific behaviors such as nictation. Graph theoretical analyses showed higher clustering of motor neurons and more feedback connections from motor to sensory neurons in the dauer connectome. Together, we propose that the dauer nervous system shares most traits of the adult nervous system and simultaneously it is remodeled for survival in challenging environments. Figuratively speaking, dauer is Peter Pan of nematodes.

779A **Alterations of the UNC-33, UNC-119, and UNC-44 Ternary Complex Negatively Impact Autophagy** Andrea Holgado, Emily Holechek, Aurora Miranda, Joseph O'Halloran, Alexa Ott, Alexia Samaro Biological Sciences, St. Edward's University

Autophagy is the process in which cellular components are degraded and recycled. Regulation of autophagy is essential for axonal development and maintenance of synapses. The ternary complex, composed of the microtubule-associated proteins (MAPs) UNC-33, UNC-44, and UNC-119, contributes to neuronal development, transportation of autophagosomes, anchoring microtubule bundles to the cortex, and inhibiting microtubule sliding. Defects in the ternary complex may contribute to the development of neurodegenerative diseases. In our laboratory, we recently began investigating the autophagy process in C. elegans mutant lacking the components of the ternary complex: UNC-33, UNC-44, and UNC-119. We hypothesize that the lack of these functional proteins will result in defects in autophagy. To test this hypothesis, we monitored autophagy flux using western blots and autophagosome maturation via confocal microscopy. To analyze autophagy flux, the levels of LGG-1/LC3 tagged with two fluorescent proteins (dFP) versus the cleaved monomeric fluorescent protein (mFP) were quantified. Autophagy was induced via starvation and compared to basal autophagy results. To examine autophagosome maturation, confocal microscopy was used to monitor autophagosomes labeled with the tandem marker mCherry-GFP-LGG-1. Preliminary analysis of western blot results shows that ternary complex mutants undergoing induced autophagy accumulate cleaved mFP resulting in unusual autophagy flux. Conversely, in basal autophagy, the accumulation of mFP was not as detectable. Results from confocal microscopy images demonstrate that mutants lacking UNC-33, UNC-44, and UNC-119 proteins have matured autophagosomes mislocalized to the neurites of neurons. These results suggest that the ternary complex may contribute to autophagic flux and cargo clearance. Microtubule sliding, a phenomenon seen in MAP mutants, may affect autophagosome transport and cargo degradation. Taken together, these findings support the role of the ternary complex in autophagy in neurons.

780A **Differential quantification of neuropeptides supports neuroregulatory & behavioral genetics** Sven Van Bael[1], Luca Golinelli[1], Amanda Kieswetter[1], Christina Ludwig[2], Liesbet Temmerman[11]KU Leuven, [2]Technical University of Munich

Animal nervous systems rely on communication via bioactive polypeptides to mediate neuro-endocrine signaling. Because neuropeptide genes are linked to dozens of physiological and behavioral processes, including important pathologies, they are actively researched. However, neuropeptide quantification is not straightforward and only very few studies have relied on it so far. This is a pity because it keeps us blind to true peptide levels and dynamics as they occur *in vivo*.

We addressed this lack of tools by developing a targeted mass spectrometry approach able to identify and quantify 263 neuropeptides of the FLP and NLP families in *C. elegans* samples. For this, we generated an in-house spectral library of 510 peptide ions based on 300 synthetic versions of worm neuropeptides and tested their behavior in LC-MS/MS. This corresponds to approximately 70% of the known and predicted neuropeptidome of *C. elegans*, which we then targeted again, now upon spiking into the biological matrix of *C. elegans* extracts. This permitted us to detect ions belonging to 88% of the target list, indicating that detection of 37 neuropeptides may be more susceptible to the sample background of a biological extract *versus* the purely synthetic sample, while 263 neuropeptides are robustly quantifiable.

Having gained the ability to target the bulk of the *C. elegans* neuropeptidome, we rely on this method to assess detailed effects of neuropeptide gene mutations, to differentially quantify neuropeptides over conditions, and to study effects of neuropeptide dosing on behavioral outcomes. Semi-quantitative peptidomics brings answering these and similar questions within reach. Scan the QR code on our poster to learn whether we can already quantify your favorite neuropeptide(s), or reach out to learn whether we may add it to our list.

781A **C. elegans behavioral screen identifies nrx-1 allele-specific small molecule modifiers** William Haury, Rebecca Kalik, Brandon L. Bastien, Michael P. Hart Genetics, University of Pennsylvania

Neurexins are synaptic adhesion molecules with diverse roles in synaptic specification, function, maintenance, and plasticity. The human NRXN1 gene encodes alpha, beta, and gamma isoforms. Deletions impacting the alpha isoform are associated with behavioral changes including autism and Tourette syndrome, while deletions impacting the other isoforms are associated with other behavioral changes including schizophrenia. Using the WorMotel chip to monitor C. elegans activity, we found nrx-1 mutants (nrx-1 is the singular ortholog of NRXN1) are unable to increase activity in response to food deprivation compared to controls. Interestingly, we identified allele-specific behavioral differences between deletion alleles lacking both isoforms versus only the alpha isoform of nrx-1. In order to perform genetic and small molecule modifier screens using behavior as a readout, we optimized a platform (WormCamp 96-well plate) to replicate the nrx-1 food deprivation phenotypes in populations of C. elegans swimming in M9 liquid. We then screened the impact of 190 small molecules in DMSO (Tocriscreen FDA-Approved Drugs Library) on the food deprivation response behavior of mutants lacking both isoforms of nrx-1. We then rescreened the top candidate small molecules in nrx-1 mutants lacking both isoforms and confirmed five molecules that significantly increased activity compared to food deprived mutants treated with DMSO vehicle alone. These five small molecules were then tested in controls (N2) and a nrx-1 alpha isoform-specific mutant allele, where we found genotype and allele-specific differences in the impact of the molecules on behavior. We are now generating dose-response curves for the top three small molecules alongside biosimilars to test for target specificity. In addition, we are screening the 190 compound library in the alpha isoform-specific nrx-1 mutant allele. We also plan to test the impact of the top small molecules on other behavioral phenotypes of mutant nrx-1 C. elegans

and dNrx-1 Drosophila. In summary, we have developed a platform to assay behavioral phenotypes in the response to food deprivation that can be used for small molecule and genetic modifier screens. Our preliminary screening identified allele-specific modifiers, supporting distinct roles and functions for NRX-1 isoforms in this behavior. Further study of the molecules and their targets will lead to mechanistic discoveries of neurexin isoform function in neuronal circuitry and behavior.

782A **Worm Neuro Atlas: A Python package combining multimodal *C. elegans* neural datasets** Francesco Randi[1], Anuj K Sharma[2], Andrew M Leifer[21]Department of Physics, Princeton University, [2]Princeton University

The number and variety of large datasets describing different aspects of the nervous system of *Caenorhabditis elegans* has recently increased dramatically. Here, we present Worm Neuro Atlas, a Python package that seamlessly integrates the following datasets: the neural Signal Propagation Atlas [1], cell-resolved transcriptome from the CeNGEN project [2], neuropeptide/GPCR interaction screens [3], monoaminergic connectome [4], anatomical connectome [5,6], estimation of chemical synapse signs [7], as well as part of the genome information from WormBase [8]. While these datasets can be accessed individually via web apps, their varied formats and interfaces make it challenging to quickly perform broad, discovery-type analysis that require access to entire datasets at once. Worm Neuro Atlas enables easy development of scripts that use these datasets to explore many different hypothesis generating questions, for example, to look for correlations between signal propagation between neurons and expression of genes across all neurons and all genes at once.

1. Randi et al., arXiv 2022
2. Taylor et al., Cell 2021
3. Beets et al., bioRxiv 2022
4. White et al., Phil. Trans. R. Soc 1986
5. Witvliet et al., Nature 2021
6. Bentley et al., PLOS Comp. Bio. 2016
7. Fenyves et al., PLOS Comp. Bio. 2020
8. WormBase, wormbase.org

783A **Negative autoregulation and Hox activation maintain critical levels of terminal selector expression** Honorine Destain[1], Paschalis Kratsios[21]Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, [2]Department of Neurobiology, University of Chicago

Across species, terminal selectors are transcription factors that establish and maintain the identity of specific neuron types throughout life. Emerging evidence suggests that, in post-mitotic neurons, the expression levels of terminal selectors must be kept within a critical range over time, yet the underlying mechanisms remain unclear. Here, we study UNC-3 (Collier/Olf/EBF), the terminal selector for cholinergic motor neurons in *C. elegans*. Studies of a human ortholog of UNC-3, EBF3, implicate the deviation of *EBF3* expression levels from a critical range in an *EBF3*-neurodevelopmental disorder. This suggests a conserved importance, from *C. elegans* to humans, for regulation of critical UNC-3/EBF levels. We find evidence for UNC-3 regulation by a combination of Hox activation and negative autoregulation. The midbody Hox proteins LIN-39 (Dfd/Scr/Hox4-5) and MAB-5 (Antp/Hox6-8), along with the Hox cofactor CEH-20 (PBX), promote expression of UNC-3. We further demonstrate evidence for negative autoregulation via a direct transcriptional mechanism, as tested by mutation of UNC-3 binding sites in the *unc-3* locus and supported by ChIP-sequencing. Using temporally controlled protein degradation, we also demonstrate that negative autoregulation occurs continuously. Finally, we show that impairing negative autoregulation results in increased adult worm swim speeds, presumably due to misregulation of motor neuron identity genes and thereby modified motor neuron function. Altogether, we uncover a molecular mechanism for the precise regulation of terminal selectors with a consequence on locomotory behavior.

784A **The thermosensory specializations of soil-transmitted parasitic nematodes** Astra S Bryant[1,2], Felicitas Ruiz[3], Joon Ha Lee[2], Michelle Castelletto[2], Elissa A Hallem[21]Physiology & Biophysics, University of Washington, [2]UCLA, [3]Microbiology, Immunology, and Molecular Genetics, UCLA

Soil-transmitted parasitic nematodes are exposed to thermal environments that vary dramatically across their life cycle. For example, the infective larvae (iL3s) of parasitic nematodes are soil-dwelling and locate hosts using body heat; the mechanistic basis of this process is poorly understood. Furthermore, the role of thermosensation in other aspects of parasite biology, including these species' ability to survive both environmental and intra-host thermal niches, is unknown.

We investigated thermosensation in parasitic nematodes using *Strongyloides stercoralis*, a potentially fatal human parasite that infects at least 610 million people globally. We found that the *S. stercoralis* thermosensory system is specialized to enable

robust host targeting. Using CRISPR-Cas9 mutagenesis and cell-type specific neuronal silencing, we found that iL3 heat seeking is dependent on a cGMP signaling pathway and sensory neuron pair that are conserved across free-living and parasitic nematodes. By imaging genetically encoded fluorescent biosensors for the first time in any non-*Caenorhabditis* nematode, we showed that *S. stercoralis* thermosensory neurons display unique thermal responses and identified the molecular substrates that underlie these properties, findings with important implications for efforts to develop new strategies for nematode control.

We also tested the thermal preferences *S. stercoralis* free-living adults, which do not host seek and must survive in the external environment, similar to the free-living, non-parasitic *Caenorhabditis elegans*. We found that the thermotaxis behaviors of *S. stercoralis* free-living adults are distinct from those of iL3s, as well as *C. elegans* adults. We tested the impact of temperature on the survival and fecundity of *S. stercoralis* free-living adults and found that warm temperatures increase fecundity while reducing lifespan, in a manner that contrasts with the ability of parasitic adults to survive exposure to host body heat. Together, these results indicate that 1) parasitic behaviors are supported by life-stage specific changes in thermal preferences and physiology, and 2) the thermal preferences of free-living *S. stercoralis* life stages are not equivalent to those of exclusively non-parasitic species. By comparing sensory function and behavior in parasitic and non-parasitic nematodes, our research addresses the fundamental question of how evolutionarily conserved neural circuits generate highly specialized behavioral repertories.

785A **The neuropeptide FLP-17 regulates a novel oviposition behavior that increases the reproductive fitness of the hermaphrodite mothers in 3D cultivation** Jin I. Lee[1], Tong Young Lee[1], Eunha Jang[1], Rocel A. Indong[1], Kyoung-hye Yoon[21]Biological Science and Technology, Yonsei University, [2]Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine

The ability for animals to adapt their behaviors to specific environments is imperative to increase their evolutionary success. This is particularly true for behaviors such as parental behaviors that directly affect reproductive fitness. Here, we identified a novel oviposition behavior in the nematode *C. elegans* that increases the survival of the young. In standard laboratory culture, the bacterivorous hermaphrodite mothers lay eggs across a 2D *E. coli* lawn with no discernable pattern. However, when cultured in a 3D NGM environment with embedded OP50 colonies they display a stereotypical behavior in which they temporarily leave the bacteria to lay eggs far away from the spherical *E. coli* colony, resulting in a scattered ring of eggs located outside the bacteria. We found that cultivation in 7% low oxygen could elicit this oviposition behavior in 2D NGM. Interestingly, we found the FMRF-like neuropeptide FLP-17 and its cognate receptor EGL-6 could regulate oviposition behavior. Unlike wild type, mutants of *flp-17* laid their eggs close to the bacterial colonies in 3D or the bacterial lawn in 2D at 7% oxygen. In addition, we demonstrate that loss of neuronal activity from the oxygen-sensing BAG neurons, and genetic ablation of the vulva muscle-controlling HSN motor neurons inhibits oviposition behavior. We show that loss of proper oviposition behavior in 3D cultivation results in lower reproductive fitness for the hermaphrodite mothers and embryonic lethality for the eggs laid in bacteria under hypoxic conditions. Finally, we show that the degree of oviposition behavior varies among wild strains of *C. elegans* found in nature. The ability for *C. elegans* hermaphrodite mothers to sense their complex 3D environments and adjust their behaviors in adverse conditions such as hypoxia may be an adaptation that has allowed the worm to thrive in diverse and often hazardous habitats.

786A **Dye-uptake of amphid neurons enhances phototaxis behavior in a LITE-1 dependent manner** Hirofumi Kunitomo, Masayoshi Kuroda, Yuichi IinoDepartment of Biological Sciences, School of Science, The University of Tokyo

Dye-filling is a simple and efficient experimental procedure commonly used to assess the integrity of sensory cilia in *Caenorhabditis elegans*. Fluorescent dyes such as 5-fluorescein isothiocyanate (FITC) and lipophilic carbocyanine dyes (DiI, DiO and analogs) are incorporated into amphid and phasmid sensory neurons through their exposed ciliated endings. Although DiI labeling does not reportedly affect cell viability or basic physiological properties of cells, the effect of dye filling on cellular and behavioral responses in nematodes remains unexplored. Here we report that dye-uptake of amphid neurons enhances light responses of the animals. *C. elegans* exhibits locomotory avoidance to short-wavelength light in an intensity- and wavelength-dependent manner. Animals avoid UV light robustly, while avoidance of blue light (typical wavelength = 480 nm) requires 10 times stronger light intensity (several mW/mm^2), and little or no response is observed to long-wavelength visible light such as yellow or red light (Ward et al., 2008, Edwards et al., 2008). Uptake of DiO (maxEx/Em = 484/501 nm) greatly enhanced the response of animals to blue light. Interestingly, DiR (maxEx/Em = 748/780 nm) conferred responsivity to red light (typical wavelength = 630 nm), to which naive animals inherently do not respond. Such dye-induced negative phototaxis depends on dye-uptake of sensory neurons as well as excitation of dyes, since the use of ciliary mutants or swapping of excitation light sources resulted in no or little response. FITC and other membrane-staining dyes also enhanced light-sensitivity of animals. DiO and DiR label the amphid neurons ASI, ADL, ASK, AWB, ASH, and ASJ. Of these, dye-uptake in ASH was sufficient for the response, whereas ablating the cell did not completely abolish it, suggesting that multiple sensory neurons are involved in the behavior. The authentic phototaxis response depends on cGMP signaling consisting of DAF-11, TAX-2 and TAX-4, as well as LITE-1, a photoreceptor protein that belongs to the invertebrate taste receptor family and mediates light response in *C. elegans*. In contrast, the dye-induced phototaxis did not require cGMP signaling nor OSM-9 TRPV channel that mediates sensory respons-

es of ASH, but was completely dependent on LITE-1. These results suggest that the signaling pathway that mediates dye-induced phototaxis partially overlaps with the authentic phototaxis pathway.

787A **Classification of *C. elegans* behaviors based on centroid movement and posture analyses** Koyo Kuze, Karin Suwazono, Midori Wakana, Moon Sun Jang, Hirofumi Kunitomo, Yu Toyoshima, Yuichi Iino Department of Biological Sciences, The University of Tokyo

To explore, navigate and avoid danger, worms move in diverse ways by coordinating muscle contraction and relaxation along their body and neck. Understanding the behavioral strategies and their control by the nervous system requires quantitative description and classification of behaviors.

To classify behavioral sequences during chemotaxis, we obtained centroid movement data from images of moving animals captured by a worm tracking system. We then applied dynamic time warping, UMAP and k-means to the movement vectors to achieve dimensionality reduction and classification of behavioral sequences. We then evaluated contribution of each group of behaviors to chemotaxis. These analyses allowed us to sub-classify seemingly similar behaviors and extract those potentially important for navigation towards chemoattractants.

We further aimed to incorporate changes of worm posture for a more in-depth behavioral quantification. Body centerline is a well-recognized representation of worm posture because worms are rod-shaped. However, we realized that existing methods fall short of precisely extracting the centerline from worm images, especially when parts of the body are attached or overlapped with each other. To address this issue, we developed a Python software called WormTracer. WormTracer finds optimal centerlines by generating worm shapes based on candidate centerlines and matching them to time-lapse worm images by gradient descent optimization. To reduce errors in the centerline identification, the optimization is performed under cost functions that evaluate smoothness of the centerlines as well as their continuity over time. By using WormTracer, we classified basal patterns of continuous worm movements and identified different sequences of basal patterns. Occurrence frequencies of these sequences and their contribution to chemotaxis were revealed.

These approaches will help deepen our understanding of worm behaviors and their regulation from a mechanical and ethological perspectives.

788A **Visualizing neuropeptide GPCR activation in *C. elegans* using PepSee** Jan Watteyne, Ellen Geens, Elke Vandewyer, Su Min Cho, Majdulin Nabil Istiban, Keertana Venkatesh, Nathan De Fruyt, Isabel BeetsKU Leuven

Neuropeptides are signaling molecules that profoundly affect the physiology and behavior of all animals. Our recent efforts in characterizing the *C. elegans* neuropeptide-GPCR interaction landscape[1] and neuropeptidergic connectome[2] have revealed dense peptidergic signaling networks that differ in structure from the wired synaptic connectome. However, the extent by which neuropeptides impinge on specific receptor-expressing cells and how these signaling patterns are shaped by context remains poorly understood. To address this, we have adopted PepSee, a genetically-encoded sensor for GPCR activation which allows the spatiotemporal visualization of neuropeptide-receptor signaling in *C. elegans*. This is achieved by release of a transcription factor tethered to the GPCR of interest specifically upon receptor activation and under blue-light illumination, which subsequently induces the expression of a nuclearly-localized fluorescent reporter. When ectopically expressed in the ASH sensory neuron, the GPCR activation sensor shows stable fluorescent readout upon exogenous application of neuropeptide ligands. Optogenetic stimulation of neuropeptide release elicits stimulation-dependent GPCR activity patterns in head and tail ganglia. Finally, GPCR activation is also observed under environments entailing endogenous neuropeptide signaling. Both multi-copy and single-copy sensor levels report GPCR activation, which can be modularly applied to different neuropeptide GPCRs. We are further using PepSee to investigate diverse spatiotemporal aspects of neuropeptide signaling. We previously found that conditional signaling of CAPA-1 neuropeptides, through activation of the neuromedin U receptor NMUR-1, underpins experience-dependent plasticity of salt chemotaxis behavior in *C. elegans*[3]. In addition, distinct cells express either *nmur-1*[4] or *capa-1* depending on the animal's food context. This highlights conditional and temporal aspects of neuropeptide signaling as important organizational motifs within the neuropeptide network, which we are further addressing with activity readouts of neuropeptide-receptor signaling. These findings and tools act as a scaffold to investigate how adaptive behaviors and physiological responses emerge from flexible neuromodulatory networks.

1) Beets *et al.* bioRxiv 2022
2) Ripoll-Sanchez *et al.* bioRxiv 2022
3) Watteyne *et al. Nat. Com.* 2020
4) Wibisono *et al. Cell Rep.* 2022

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789A **Studying CEPsh glia uncovers factors of early development and lifelong maintenance of astroglia architecture** Francesca Coraggio[1], Francesca Caroti[1], Mahak Bhushan[2], Spyridon Roumeliotis[2], Carlo Bevilacqua[2], Carlo Bevilacqua[2], Robert Prevedel[2], Georgia Rapti[11]Developmental Biology, European Molecular Biology Laboratory, [2]European Molecular Biology Laboratory

Faithful circuit function relies on proper formation and interactions of neurons and glia, both vital circuit sculptors. Despite extensive neurodevelopment studies, mechanisms of morphogenesis and maintenance of glial cell architecture remain understudied. The *C. elegans* nervous system offers a powerful setting to tackle these questions, due to its stereotyped anatomy, glia identified in single-cell resolution and largely dispensable for neuronal viability. We study the architecture of CEPsh glia, combining genetic screens, quantitative imaging of cellular, subcellular features and extracellular matrix (ECM), with genetic manipulation of glial-cells and their environment.

We and others previously showed that CEPsh glia have architecture, functions and molecular content analogous to radial glia and astrocytes. They form early, axon-coalescing, non-branching processes to initiate circuit assembly, which transform into elaborate, tufted membranes ensheathing synapses. Adding to this transformation, they express factors enriched in vertebrate radial glia and astrocytes. In candidate-gene and unbiased screens we isolated mutants harboring CEPsh glia with abnormal morphology. We study them to dissect the implicated (extra)cellular factors and their mechanisms of action. Our work uncovers factors of development and maintenance of glia architecture. We will report our progress in these directions.

Neural circuits are susceptible to environmental changes that challenge tissue integrity. Glial cell interactions define connectivity, thus their architecture should be maintained throughout life and failure to preserve their integrity results in compromised circuit function and neuropathology. Therefore, dissecting the maintenance of glia architecture is crucial to understand lifelong circuit integrity. We isolated mutants with age-progressive, environment-dependent defects in glial architecture and a consequent disruption of axons and synapses. We uncover that disrupting the heat-shock chaperone system in epithelial cells causes ECM disruption and altered animal biomechanics. These changes result in the disruption of CEPsh glia integrity and their hypersensitivity to temperature and mechanical stress. Furthermore, we show that modifying ECM content, cell junctions, animal mechanics or the environment safeguards glial integrity. This fine interplay between proteostasis, ECM and mechanics, ensures lifelong robustness of glial architecture.

790A **Inter-tissue regulation of neuromuscular function by the G protein-coupled receptor FSHR-1** Morgan Buckley, Will Jacob, Letitia Bortey, Alyssa Ritter, Amy Godfrey, Sarah Olofsson, Allyson Munneke, Julie Kolnik, Ryan Adkins, Lauryn Padgett, Makenzi McClain, David Emch, Kyle Cherry, Lilly Rademacher, Tanner Kutoloski, Barry Wei, Alexandra Alva, Abigail Screen, Jennifer Kowalski Biological Sciences, Butler University

Cross-tissue communication, including inter-neuronal, glial-neuronal, and gut-brain, is critical for neuronal signaling balance and nervous system function in varied physiological conditions. G protein-coupled receptors (GPCRs) are a group of transmembrane proteins expressed in most tissues and involved in multiple aspects of neuronal signaling and in coordinating multi-tissue responses to stimuli. FSHR-1 is the sole *Caenorhabditis elegans* ortholog of mammalian glycopeptide hormone GPCRs and was identified as a regulator of neuromuscular signaling balance and resistance to oxidative and other stresses. Mutations in mammalian *FSHR* are implicated in depression and affective disorder phenotypes also linked to oxidative stress, but the mechanisms are unknown. Inhibition of *fshr-1* expression in *C. elegans* was reported to cause reduced muscle contraction and accumulation of fluorescently labeled synaptic vesicles in excitatory cholinergic motor neurons. We used aldicarb paralysis and swimming assays to confirm the neuromuscular defects of *fshr-1*(*ok778*) loss-of-function (*lf)* mutants, which are exacerbated in aged animals and in those exposed to chronic oxidative stress. Using quantitative imaging, we found that, in addition to a build-up of cholinergic synaptic vesicles, *fshr-1* mutants accumulate UNC-10/RIM and SYD-2/Liprinα active zone proteins. Epistasis analyses further indicate *fshr-1* acts upstream of *gsa-1/GaS*, *acy-1*/adenylyl cyclase*,* and the lipid kinase gene, *sphk-1,* and downstream of genes encoding the putative glycopeptide ligands, *flr-2/GPA1* and *gplb-1*/*GPB5,* to control neuromuscular function. Despite the effects of *fshr-1*(*lf*) on cholinergic motor neurons and neuromuscular behaviors, cell type-specific knockdown, rescue, and overexpression experiments demonstrate intestinal *fshr-1* is both necessary and sufficient for neuromuscular function, and *fshr-1* re-expression in other *fshr-1*-expressing tissues, including neurons and glia, also restores the neuromuscular behaviors of *fshr-1* mutants. These data suggest *fshr-1* exerts its neuromuscular effects, at least in part, through cell non-autonomous mechanisms and support an inter-tissue model of regulation in which activation of FSHR-1 in the intestine and/or in other distal tissues causes release of signals that maintain cholinergic vesicle release and muscle excitation. Current work is aimed at determining the mechanisms and nature of this distal signal secretion under physiological and oxidative stress conditions.

791A **Caught in the act: visualizing sex-specific neuroblast divisions in the ventral cord** Jennifer R Wolff[1], Collin Adams[1], Emma Carlson[1], Jisoo Yeom[1], Andrea K Kalis[21]Biology, Carleton College, [2]Biology, St. Catherine University

For neurons to develop and function sex-specifically, they must integrate both sex-shared and sex-specific developmental cues. *C. elegans* sex-specific ventral cord neurons (VCNs) arise from Pn.aap neuroblast cells, which divide in males to generate nine CA/CP neuron pairs, and either die or differentiate in hermaphrodites to produce the six VC neurons. Differentiation, division, and survival of sex-specific VCNs depends on the coordinated activities of Hox transcription factors LIN-39 and MAB-5. We have previously shown that LIN-39 and MAB-5 act together at cis-regulatory modules to regulate neuronal gene expression, resulting in regionalized anteroposterior differentiation among CPs. However, how sexual regulators and Hox proteins intersect to influence early events in male-specific neurogenesis, such as the division of Pn.aap, has remained elusive.

The Pn.aap division in males occurs in L3, after all other P-derived VCNs have ceased division; this has allowed us to use a cell cycle sensor, *mcm-4pro::cdksensor::gfp,* to examine early sex-specific neurogenesis. We find that *mcm-4pro::cdksensor::gfp* is expressed in nine nuclei beginning in late L2 males. This GFP expression becomes cytoplasmic in early-mid L3 indicating cell cycle commitment, and these cells divide in mid-late L3 and continue to express GFP in nine pairs of nuclei through L4. This marker will allow us to investigate *mab-5* and *lin-39* expression in conjunction with Pn.aap division, and the influence of *mab-5* and *lin-39* mutations on the Pn.aap cell cycle. We are currently developing an mKate2 version of the cell cycle sensor to use in combination with N-terminally-mNeon-tagged *lin-39* and *mab-5* reporters.

To further investigate sex-specific Pn.aap division, we have created a strain in which the P lineage has been selectively masculinized with an *hlh-3pro::fem-3::mCherry* transgene. In XX *hlh-3pro::fem-3::mCherry* worms, mCherry is expressed in all VCNs from L1 through adulthood. Strikingly, Pn.aap neuroblasts assume a male-typical pattern of development in these hermaphrodites: Pn.aap remains in the cell cycle and divides during L3, with posterior daughters differentiating as serotonergic neurons, a fate normally reserved for male CPs. Further study of masculinized neurogenesis in the context of an otherwise female soma 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.

792A **Uncovering new GABA transporters in *C. elegans* using knock-out strains and classical immunostaining** Nalia Samba[1], Elise Cheynet[2], Marie Gendrel[11]IBENS - ENS - CNRS - INSERM - PSL university, [2]MeLis, CNRS UMR 5284, Université Claude Bernard Lyon 1

Functional neuronal circuits rely on excitation and inhibition balance, which is associated with many neurological diseases when dysregulated (schizophrenia, depression etc…). Our work focuses on GABA, the main inhibitory neurotransmitter in mature neurons. Traditionally in *C. elegans*, 26 out of 302 neurons were identified as GABAergic based on the co-expression of three determinant proteins: 1) GAD/UNC-25, which synthesises GABA from glutamate, 2) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and 3) GAT/SNF-11, a plasma membrane transporter that recaptures GABA from the synaptic cleft. With improved anti-GABA immunostaining, 15 additional GABA-positive neurons have been identified. These neurons do not all coexpress the three determinant proteins. More specifically, three pairs of neurons express neither GAD*/unc-25* nor VGAT*/unc-47* nor GAT*/snf-11*. Thus, they are unable to synthesise, uptake or package GABA the way we know it. Moreover, they are known to have postsynaptic partners expressing $GABA_A$ receptors. This strongly suggests that they release GABA by mechanisms that have yet to be identified.

We hypothesise that these neurons use other transporters for uptake and vesicular packaging of GABA. We aim at identifying them and to probe 53 putative amino acid transporters.

In this part of the project, we study how null alleles of these genes can affect anti-GABA immunostaining. The mutation of either a membrane or a vesicular transporter should decrease or increase the staining, respectively. To this date, 31 out of the 35 mutant strains available in the lab have been analysed. We have found 5 candidate genes that affect the anti-GABA staining. In order to confirm these results, we are generating null alleles using the CRISPR/Cas9 technology and assessing the expression pattern of the 5 candidate genes. For the validated ones, we will then assess GABA transport in ectopic cell culture and/or using xenopus oocytes.

CRISPR/Cas9 will also be used to generate null alleles for genes with no available mutant strain.

793A **Functional recovery associated to dendrite regeneration in PVD neuron** Harjot Kaur Brar, Swagata Dey, Pallavi Singh, Anindya Ghosh Roy National Brain Research Centre

PVD neuron of *C. elegans* is a highly polarized cell with a well-defined axonal and dendritic compartments. PVD neuron operates on multiple sensory modalities including harsh touch sensation and proprioception (Tao et al., 2019). Although both axon and dendrites of this neuron show regeneration response following laser-assisted injury (Brar et al., 2022), it is unclear how behaviours associated to this neuron are affected by axotomy and dendrotomy. It is also unclear whether neurite regrowth following injury would lead to functional restoration. Upon axotomy using femtosecond laser, we saw that the harsh touch

response was specifically reduced while the body posture was unperturbed. Subsequently, axon regrowth is highly correlated to recovery in the harsh touch response defect, which was dependent on DLK-1 MAP Kinase pathway.

Dendrotomy of both major and minor primary dendrites affected the wavelength and amplitude of sinusoidal waves formed by the body posture of the worm without any apparent effect on harsh touch response. Furthermore, dendrite regeneration also correlated to the restoration of body posture characteristics. Our data demonstrated that the axon and dendrites differentially regulate functions associated with PVD neurons. It also revealed that dendrite and axon regeneration are both functionally and molecularly distinct.

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794A ***sma-1* mutants have a reduced response to electricity** Victoria Lam[1], Annalise Totten[1], Ling Fei Tee[2], Mariyam Muhammad[3], Julia Rosecrans[3], Estefany Rivera-Hernandez[1], Haifa Al-Jubari[3], Eulia Keomany[3], Kotarou Kimura[2], Jared Young[11]Mills College at Northeastern University, [2]Nagoya City University, [3]Mills College

In response to alternating current of 30V applied to the agar substrate, *C. elegans* increases movement speed (Tee et al., in revision). This response could be a manifestation of a fear emotion in the worms, causing them to panic and attempt to escape. To investigate the mechanisms underlying this behavior, a forward genetics screen was conducted. EMS mutants were screened for a reduced response to electricity. One of the mutants isolated from the forward screen was sequenced and its sequence was compared to the background N2 strain genome. The likely region of the causative mutation was mapped to be on chromosome V and sma-1 was identified as a candidate gene, partly based on the visible phenotypes of the isolated mutant, which resemble those of *sma-1* mutants. Three alleles of *sma-1* (e934, e30, and ru18) were tested for electricity response and all were found to be defective. On average, these *sma-1* worms showed less increase in their movement speed compared to wild-type worms. We plan to test hypotheses regarding the specific biological contribution of SMA-1 to the electricity response. Phenotypes of greatest interest associated with *sma-1* mutants are small size and defective excretory canals.

795A ***sut-6/NIPP1* modulates tau toxicity** Rebecca L Kow[1,2], Brandon P Henderson[1], Aristide H Black[1], Brian C Kraemer[1,2,3,-4]1Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, [2]Division of Gerontology and Geriatric Medicine, University of Washington, [3]Department of Psychiatry and Behavioral Sciences, University of Washington, [4]Department of Laboratory Medicine and Pathology, University of Washington

Neurodegenerative diseases exhibiting the pathological accumulation of tau such as Alzheimer's disease and related disorders still have no disease-modifying treatments and the molecular mechanisms of neurodegeneration remain unclear. To discover additional suppressor of tauopathy (*sut)* genes that mediate or modulate the toxicity of pathological tau, we performed a classical genetic screen using a tau transgenic *C. elegans* model. From this screen we identified a c.876G->A change in the *C. elegans* gene *B0511.7*, which we subsequently refer to as *sut-6.* This mutation results in the coding change of W292X, introducing a premature stop codon 11 amino acids before the normal C-terminus of the protein. *sut-6* is the *C. elegans* homolog of mammalian *NIPP1,* and based on homology, the mutation is the predicted to truncate the C-terminal domain important for RNA binding. Using CRISPR based genome editing approaches, we generated null and additional C-terminally truncated alleles in *sut-6* and found that loss of *sut-6* or *sut-6(W292X)* suppresses tau-induced behavioral locomotor deficits, tau protein accumulation, and neuron loss. Neuronal overexpression of SUT-6 protein did not significantly alter tau toxicity, but neuronal overexpression of SUT-6 W292X mutant protein reduced tau-mediated deficits. In summary we have shown that *sut-6/NIPP1* modulates tau toxicity and found a dominant mutation in the RNA binding domain of *sut-6* which strongly suppresses tau toxicity. This suggests that altering RNA-related functions of SUT-6/NIPP1 instead of complete loss of SUT-6/NIPP1 will provide the strongest suppression of tau.

796A **A statistical analysis workflow for multivariate behavioural data in *C. elegans*** Daniel-Cosmin Marcu[1], Emanuel Busch[1], Melanie Stefan[21]Institute for Mind Brain and Behavior, Health and Medical University, [2]Medical School Berlin

The locomotor behaviour of the nematode *Caenorhabditis elegans* is a complex response to environmental and physiological conditions. To survive, *C. elegans* needs not only to move, but also to adjust and adapt aspects of its movement such as speed in response to changing conditions through locomotory plasticity. It is poorly understood how external factors such as oxygen concentration, the overall chemical composition of an environment, or previous experience interact dynamically with internal

factors such as age, developmental stage, or genetic background. Teasing apart the contribution of these complex interacting factors to animal behaviour requires the acquisition of multidimensional data. This type of data can be challenging to understand and analyse by experimenters with limited experience in programming or statistics. We are developing an analysis workflow that allows users to identify the appropriate types of statistical tests to perform on complex, multivariate behavioural datasets in order to understand the correlation and dependencies between environmental and physiological variables. Our pipeline provides strategies for cleaning, formatting, and visualising data prior to analysis, steps for performing a statistical analysis, and options for generating figures and reports for publication. It supports versatility in experimental designs and can become an essential tool in studies of ageing, behavioural plasticity, or neurogenetics.

797A **Hox factors collaborate with or antagonize terminal selectors to generate neuronal diversity within the *C. elegans* ventral nerve cord** Manasa Prahlad[1], Paschalis Kratsios[21]Committee on Genetics, Genomics and Systems Biology, University of Chicago, [2]Department of Neurobiology, University of Chicago

All nervous systems use a great diversity of cell types. Classification of neuron types often considers functional, morphological, and molecular criteria. But neuronal subtype diversity is an understudied facet of nervous system development. The *C. elegans* ventral nerve cord (VNC, analogous to vertebrate spinal cord) possesses 53 cholinergic and 19 GABAergic motor neurons (MNs), grouped into 8 anatomically distinct types, offering a prime model to study neuronal diversity. Cholinergic neurons of all types, irrespective of their cell body position in the VNC, rely on the terminal selector UNC-3 (Collier/Olf/Ebf) to promote the expression of genes critical to their function (e.g., neurotransmitter (NT) synthesis, NT transport). Similarly, GABAergic neurons require UNC-30 (Pitx). Intriguingly, our single-cell RNA-seq of VNC MNs, generated in collaboration with the Miller lab at Vanderbilt University, shows a new layer of diversity within MNs of all 8 types: MNs in different regions of the VNC are molecularly distinct, i.e., molecular profiles of individual MNs differ based on their cell body positions. The gene *mig-13*, encoding a protein crucial for the migration of some neuroblasts, is an entry point to study how antero-posterior (A-P) MN subtype diversity is generated along the VNC: *mig-13* is expressed in most MNs located anterior to the vulva, but almost none posterior. We hypothesized that its region-specific expression pattern was due to Hox activity. Indeed, genes for the anterior Hox *ceh-13* (Lab/Hox1), midbody Hox *lin-39* (Scr/Dfd/Hox4-5), and Hox cofactor *ceh-20* (Exd/Pbx) are all required for normal *mig-13* expression in anterior VNC MNs. Similarly, the posterior Hox gene *egl-5* (Abd-B/Hox9-13) is essential for *mig-13* expression in MNs of the pre-anal ganglion. UNC-3 and UNC-30 also activate *mig-13* expression in anterior VNC MNs. These results show that terminal selectors collaborate with distinct Hox factors to promote expression of terminal identity features in MNs along the VNC.

Interestingly, we also observed antagonism between these terminal selectors and the posterior midbody Hox, *mab-5* (Antp/Hox6-8). Without *mab-5*, *mig-13* is derepressed in MNs posterior to the vulva. This derepression is dependent on terminal selector activity, indicating MAB-5 antagonizes the activator function of terminal selectors. In sum, our findings reveal how Hox factors and terminal selectors intersect to generate neuronal diversity along the A-P axis of the nervous system.

798A **Dissecting the *Caenorhabditis elegans* exploratory head movements** Pinjie Li[1,2], Quan Wen[1,21]Chinese Academy of Sciences Key Laboratory of Brain Function and Diseases, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China, [2]Hefei National Laboratory for Physical Sciences at the Microscale, Center for Integrative Imaging, University of Science and Technology of China, Hefei, China

When navigating in a natural environment, the head of *Caenorhabditis elegans*, bombarded with various sensory stimuli, exhibits irregular and unpredictable movements. While such exploratory behaviour is well known among *C. elegans* researchers, (1) systematic quantitative analysis of head bending activity is scarce; (2) how the underlying motor circuit generates chaotic bending dynamics is unclear; (3) the influence of external sensory inputs remains largely unknown. Here we report recent progress in the three directions. First, by applying variational mode decomposition (VMD), we find that *C. elegans* head movements can be divided into high-frequency swings and low-frequency sinusoidal bending activity back-propagating along the body. Second, combinatorial optogenetics on cholinergic motor neurons and phase space analysis reveal that different types of head motor neurons play different roles in the head bending dynamics: RMD contribute to irregular and high-frequency head swings; SMD are responsible for maintaining the ventral and dorsal bending states; SMB generate low-frequency rhythmic oscillation and facilitate head-body coupling. The three types of motor neurons are necessary components of the central pattern generator in the *C. elegans* head motor circuit. Third, we discover that global inhibition of sensory inputs, surprisingly, increases the irregularity of head bending activity. We recapitulate these observations with a neural dynamic model, highlighting the findings that different types of excitatory motor neurons, despite sharing common motifs of functional connectivity, can take charge of different aspects of head exploratory dynamics in *C. elegans*.

799A **Identification of novel genetic interactions between RNA-binding proteins regulating neuronal development and animal fitness** Sharanja Premachandran, Alison Nguyen, Una McNally, Luca Savo, Welna He, John Anthony Calarco Cell and Systems Biology, University of Toronto

As precursor messenger RNAs (pre-mRNAs) are transcribed, they become decorated by a host of RNA-binding proteins (RBPs), influencing their metabolism and ultimately their fates. These RBPs are often underappreciated for their multivariate roles in gene expression, including the mechanisms of splicing, polyadenylation, export, localization, and degradation. While much attention has been paid to the roles of transcription factors in establishing cell fate and function, recently, RBPs have emerged as key players in development and disease. However, owing to the complex and combinatorial action of RBPs on their target mRNAs, many single gene functional analyses do not reveal discernible phenotypes. Consequently, the functions of the majority of RBPs have yet to be characterized. Using CRISPR/Cas9-generated marked deletion alleles, I have employed a combinatorial null allele approach to explore the function of 24 neuronally-enriched RBPs through two parallel reverse genetic screening approaches in *Caenorhabditis elegans*: (1) high-throughput relative fitness assays, and (2) PVD neuron dendritic arborization screens. To date, these screens have identified 16 novel candidate genetic interactions between RBP pairs. Of particular interest is a novel genetic interaction between *mbl-1* and *unc*-75, wherein a branching defect of PVD dendrites is observed at all orders of branching in the double mutant and is especially pronounced at the anterior aspect of animals. Additionally, I have identified defects in single RBP gene mutants that perturb the stereotypic branching of dendritic arbors observed in wild-type animals. This includes novel identification of defects in sister dendrite self-avoidance—a mechanism by which dendrites of the same neuron form non-overlapping branches to cover the receptive field. Thus, my research supports the roles of RBPs in neurite development—raising questions about the combinatorial and spatiotemporal regulation of neuronal mRNA targets. Together, these screens will guide transcriptome analysis to characterize the regulatory roles of candidate RBPs in the multitude of signaling pathways implicated in neurite outgrowth and retraction, which may become perturbed in disease.

800A **Functional recovery during regeneration of a head ganglion neuron in *C. elegans*** Yuki Tsukada[1], Ikue Mori[21]Department of Biosciences and Informatics, Keio University, [2]Nagoya University

The properties of central nervous system (CNS) regeneration remain largely unknown compared to peripheral nervous system (PNS) regeneration. In *C. elegans*, while the regeneration of mechanosensory and motor neurons at the ventral nerve code resembling the PNS have been studied, there are few reports about the regeneration of head ganglion neurons resembling the CNS.

Here, we report distinct regeneration patterns and functional recovery of the head ganglion neuron AFD. We cut the proximal region of the axon at the adult stage using a femtosecond laser and observed the resultant neuron with a confocal microscope. The thermo-sensory neuron AFD shows a variety of regeneration patterns such as sprouting, branching, and wandering. Although the cutting position is fixed with a proximal region of the target axon, sprouting positions varied in the axon, soma, and dendrite. Most regenerating axons extended toward the nerve ring region where the distal part of the cut axon is located, implicating the existence of the guidance cues specifically toward the nerve ring. Interestingly, even the regenerated neurite sprouting from soma or dendrite directed to the nerve ring and made connections with the distal part of the axons. Moreover, AWC, which is previously reported to show no regeneration, exhibited extension of the neurite from the dendrite to the nerve ring. We confirmed the functional recovery of the reconnected neurons for information transmission from AFD to postsynaptic partner AIY by calcium imaging after the regeneration. Thus, proximal axonal cuts in adulthood induced robust regeneration to recover the functional connection. On the other hand, laser surgery of the nerve ring region did not show any regeneration at all. Killing CEPsh cells by caspase resulted in inhibiting regeneration from the dendrite and rather promoting regeneration from the axon, suggesting that CEPsh cells are important to distinguish between dendritic and axonal regeneration. We are exploring molecular mechanisms underpinning the observed head neuronal regeneration and found that PKC-1 may play a role in inhibitory mechanisms for sprouting neurite in the regeneration of AFD neurons.

Our study clarifies the manner of functional and morphological recovery in head ganglion neurons, providing a model system to understand functional CNS recovery with a powerful genetic and physiological approach using *C. elegans*.

801A **The gene *igeg-1* encodes a Neuregulin-like EGFR ligand** Andrew J Hill[1], Bryan Robinson[2], Jesse G Jones[2], Paul W Sternberg[1], Cheryl Van Buskirk[21]Biology and Biological Engineering, California Institute of Technology, [2]Biology, California State University-Northridge

In *C. elegans*, exposure to damaging conditions triggers a programmed state of behavioral quiescence known as stress-induced sleep or sickness sleep (SIS) that is dependent on the Epidermal Growth Factor Receptor LET-23/EGFR. Though LIN-3 is the sole recognized EGF family ligand in *C. elegans*, and LIN-3 ectopic overexpression can trigger sleep, we present our surprising finding that LIN-3 is not the endogenous ligand used during SIS. In a genetic screen for SIS-defective mutants we identified IGEG-1, a transmembrane protein with extracellular immunoglobulin (IG) and EGF domains, similar to vertebrate Neuregulins NRG1 and NRG2. We find that IGEG-1 functions genetically upstream of LET-23/EGFR and that substitution of a conserved cysteine within the EGF domain phenocopies an IGEG-1 deletion, suggesting that IGEG-1 is a functional EGFR ligand. In contrast to *lin-3* mutants, *igeg-1* mutants are viable and fertile with normal vulval development, and they show a penetrant loss of SIS. Ectopic

overexpression (OE) of IGEG-1 promotes robust behavioral quiescence that is dependent on the sleep-promoting ALA neuron. Like other EGF ligands including LIN-3, IGEG-1 is produced as a transmembrane proprotein that requires processing within the juxtamembrane region to initiate paracrine EGF signaling. We find that the sleep-promoting activity of IGEG-1(OE) - but not of LIN-3(OE) - requires the ADM-4 metalloprotease, which is orthologous to the vertebrate stress-responsive sheddase ADAM17. Consistent with a role for ADM-4 in IGEG-1 shedding, the requirement for ADM-4 in sleep can be bypassed with a constitutively secreted form of IGEG-1. While LIN-3 is under tight transcriptional control, both ADM-4 and IGEG-1 are widely expressed. To identify the site of IGEG-1 release during SIS we examined the tissue-specific requirements for ADM-4, which is predicted to function cell-autonomously. We found that the ADM-4 site of action depends on the type of damage that is used to trigger sleep, supporting a model in which IGEG-1 is released from the damaged tissue(s). Our studies indicate that LIN-3 is not the only functional EGF family ligand in *C. elegans*, and that IGEG-1 is shed by ADM-4 in response to cellular damage to promote EGFR-dependent sleep.

802A **Updates from the OpenWorm project: incorporating NeuroPAL data and ASH neuron electrophysiological recordings** Padraig Gleeson[1,2], Bradly Alicea[2,3], Matteo Cantarelli[2,4], Giovanni Idili[2,4], Chee Wai Lee[2], Andrey Palyanov[2,5], Sergey Khayrulin[2,5], Tom Portegys[2], Vahid Ghayoomie[2], Stephen Larson[2,41]Department of Neuroscience, Physiology and Pharmacology, University College London, [2]OpenWorm Foundation, [3]Orthogonal Research and Education Laboratory, [4]MetaCell, [5]A.P. Ershov Institute of Informatics Systems, Siberian Branch of the Russian Academy of Sciences

The OpenWorm project (http://openworm.org) is a global, open science collaboration of computational and experimental neuroscientists, software engineers, and volunteers. Its overarching goal is to create a comprehensive cell-by-cell computer model of C. elegans that can replicate the animal's behavior in detail, thus consolidating anatomical and physiological knowledge of this model organism and elucidating the underlying mechanisms of how behavior is generated by a complete nervous system.

Significant milestones have been achieved in this endeavor, including the development of Sibernetic, a simulation engine that models the environment and the 3D body of the worm, and Geppetto, a web-based visualization and simulation engine that enhances the accessibility of the project's output. Frameworks such as c302 for generating network models of C. elegans neurons and owmeta for accessing and sharing anatomical and physiological data have also been developed. In addition, the DevoWorm subproject focuses on embryogenesis and the study of comparative development between C. elegans and various vertebrate and invertebrate species.

Recently, the project has leveraged data from the NeuroPAL technique, a revolutionary genetic strain of C. elegans that labels each neuron with a specific fluorescent marker of a different color. This allows for easier identification of neurons across experiments and animals, thereby improving the ability to track and study individual neurons in detail. The incorporation of NeuroPAL data into computational models of C. elegans has further enhanced the project's data about the worm's nervous system.

Furthermore, the project has converted electrophysiological recordings of the ASH neuron, made by the Wormsense Lab of Miriam Goodman, to open, standardized Neurodata Without Borders (NWB) format. This conversion has made the data more accessible to researchers and has enabled the incorporation of the ASH neuron's activity into biologically realistic computational models of C. elegans.

The new OpenWorm Studentship program incentivizes junior researchers to contribute their work to the project and collaborate on creating biologically realistic computational models of C. elegans.

We present these recently incorporated datasets as well as organisational updates to the OpenWorm project, which will help advance our long-term goal of creating a comprehensive and detailed simulation of a living organism's nervous system and behavior.

803A ***C. elegans* effort-versus-reward studies enabled by a novel microfluidic arena** Muneki Ikeda[1], Yizhou Chen[2], Xaq Pitkow[2,3,4], Saul Kato[11]Department of Neurology, Weill Institute for Neurosciences, University of California San Francisco, [2]Department of Neuroscience, Baylor College of Medicine, [3]Department of Electrical and Computer Engineering, Rice University, [4]Center for Neuroscience and Artificial Intelligence, Baylor College of Medicine

Understanding the cognitive processes of speechless animals, such as *C. elegans*, has been attempted by monitoring their behavior. These attempts commonly rely on the assumption that animals are maximizing objective functions such as net reward, economic utility, or environmental information. However, animals do not always exhibit such optimal decision-making. One reason

is that they do not have access to the complete and accurate properties of the outer world; instead, they generate an internal subjective model of the world from limited observation, resulting in suboptimal decision-making. Thus, to accurately predict the cognitive processes of animals from their behavior, it is crucial to infer their internal model of the outer world.

Inverse rational control (IRC) is an approach to estimating an agent's internal model of the world. It posits a reward function, which is estimated alongside the internal model, by maximizing the likelihood of measured behavior, i.e. the agent's sensory observations and actions. Therefore, to apply IRC to animal behavior, it is necessary to monitor the real-time sensory stimuli experienced by animals, as well as their real-time behavior. This is challenging even in *C. elegans* studies, as the spatiotemporal distribution of stimuli can be difficult to monitor in classical behavioral assays.

In this study, we developed a microfluidic arena system that allows hundreds of worms to move freely through a heterogeneous mechanical environment while controlling stimuli distribution and recording individual animal posture, modified from an established arena that worms navigate by crawling. The arena is 2 cm x 2 cm x 70 µm with several thousand circular posts of various diameters arrayed at 100 µm center-to-center distances. Fluid continuously flows from inflow channels to outflow channels within the arena, and we can control chemical concentration distribution by selecting the fluids' composition and varying the design of the inflow channel structure. Within an arena whose distance between posts gradually varies from 1 µm to 100 µm, we found that worms maximally accumulated where the inter-post spacing is 35 µm. We then found that the application of a gradient of chemical attractant reliably shifted this preference location, suggesting that the worms were balancing effort and reward. By controlling chemical stimuli, mechanical stimuli, and the combination of both, we apply IRC to estimate how the worm's internal model matches the outer world, as a basis for understanding normative cognitive processes.

804A **Tale of two behaviors: Sleep and Memory** Rashmi Chandra[1], Fatima Farah[2], Fernando Muñoz-Lobato[1], Anirudh Bokka[3], Kelli Benedetti[1], Chantal Brueggemann[1], Mashel Saifuddin[1], Julia Miller[1], Joy Li[3], Eric Chang[3], Aruna Varshney[3], Vanessa Jimenez[3], Anjana Baradwaj[3], Cibelle Nassif[3], Sara Alladin[3], Kristine Andersen[3], Angel Garcia[1], Veronica Bi[3], Sarah Nordquist[1], Raymond Dunn[1], Kateryna Tokalenko[3], Emily Soohoo[3], Vanessa Garcia[3], Sukhdeep Kaur[3], Malcolm Harris[3], Fabiola Briseno[3], Brandon Fung[3], Andrew Bykov[3], Hazel Guillen[3], Decklin Byrd[3], Emma Odisho[3], Bryan Tsujimoto[3], Alan Tran[3], Alex Duong[3], Kevin Daigle[1], Rebekka Paisner[1], Carlos Zuazo[1], Matthew Churgin[4], Christopher Fang-Yen[4], Martina Bremer[5], Saul Kato[6], Miri VanHoven[3], Noelle L'Etoile[11]Cell and Tissue Biology, University of California San Francisco, [2]San Jose state University, [3]Biological Sciences, San Jose state University, [4]Bioengineering, University of Pennsylvania, [5]Department of Mathematics and Statistics, San Jose state University, [6]Department of Neuroscience, University of California San Francisco

Sleep and memory, both reveal the cognitive health of an animal and are conserved across species. However, how sleep benefits memory remains unknown. We show that though the *Caenorhabditis elegans* nervous system has a limited number of neurons, sleep is still necessary and sufficient to promote memory. We show that not all sleep-inducing neurons are required in forming long term memory, for example, the sleep promoting ALA neuron is required to induce post training sleep and preserve long term memory, but the sleep promoting RIS neuron is dispensable. This may indicate that specific interactions between neurons may allow sleep to benefit memory. We hypothesized sleep implements circuit specificity through brain-wide functional modifications and/or through circuit specific structural modifications.

We found memory consolidation, but not acquisition, requires a pair of interneurons, the AIYs, which play a role in odor-seeking behavior. In worms that consolidate memory, post training sleep is required to diminish inhibitory synaptic connections between the AWC chemosensory neurons and the AIYs. This suggests that sleep directs specific structural alterations that lead to memory. Thus, we demonstrate in a living organism that sleep is required for events immediately after training that drive memory consolidation, require specific neurons, and alter synaptic structures.

To understand how sleep might promote cognitive health and memory, we will measure calcium transients in animals that express GCaMP6f in the nuclei of all neurons in the animal. By understanding how the activity patterns of all neurons (the brain state) changes in different forms of sleep such as during post training sleep and/or during heat shock induced sleep, we may identify sleep specific circuits that serve different purposes. For example, recovery from heat shock may promote a distinct brain state when compared with recovery from training. Thus, by identifying the neuron ensembles that promote post-training sleep versus heat shock induced sleep, we will trace the circuit through which sleep acts to benefit memory.

Sleep deprivation and memory loss are the two most common cognitive impairments that accompany neurodegenerative diseases. This project will identify if sleep manipulation can delay the onset and/or limit the progression of cognitive deficits observed in neurological diseases that entail dementia.

805A **Investigating the role of CEP sheath glia in long term memory** Angel J Garcia[1], Rashmi Chandra[1], Evangeline Chien[1], Raymond L Dunn[1], Fatema Saifuddin[1], Julia Miller[2], Noelle D L'Etoile[11]Cell and Tissue Biology, UCSF, [2]UCLA

Experience-dependent learning and memory are vital to our ability to survive in diverse conditions. Uncovering the cellular and molecular mechanisms behind memory consolidation will guide therapeutic development for memory loss, a typical symptom of aging and neurodegenerative disease. Studies in mammals show that glial cells, the support cells of the nervous system, may play important roles in memory. However, the complexity of the mammalian brain makes it difficult to trace memory to specific neural circuits and to identify the cellular interactions between glia and neurons that facilitate memory consolidation.

Simple organisms, such as the nematode *Caenorhabditis elegans*, can learn and retain memory. Through subjecting the *C. elegans* to a training regime that pairs an innately attractive odor with a negative stimulus, we show that they can be trained to be repulsed by that same odor. We can then quantify the worms' memory over time using a simple behavioral assay. As the neural circuits of *C. elegans* are completely mapped out, we can examine how glial cells impact specific neural circuits linked to memory. *C. elegans* have a variety of glia, such as the CEP sheath (CEPsh) glia, which resembles vertebrate astrocytes in morphology, function, and expression of homologous genes. We ask, do CEPsh glia play a role in long-term memory, and if so, what are the mechanisms behind it?

To probe the glia-memory relationship, we have expressed the plant-derived protein, miniSOG, in the CEPsh glia of the worm, which inactivates the glia when exposed to blue light through the generation of reactive oxygen species. We found that if the CEPsh glia is inactivated during memory consolidation, the animals do not retain memory. Whole-brain calcium imaging of the worm shows that inactivation of CEPsh cells results in wide-scale neuronal disruption of calcium activity. Further experiments are being conducted to investigate specific proteins that are crucial to the learning and memory pathways.

806A **Social regulation of maternal provisioning via defined neurocircuits** Jadiel Wasson[1], Gareth Harris[2], Yun Zhang[3], Susan Mango[41]Biozentrum, University of Basel, [2]California State University, Channel Islands, [3]Harvard University, [4]Biozentrum

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 to and interpreted by the embryo remain largely unknown. Recently, we have uncovered an alternative mode of information transfer from mother to embryo in C. elegans. Here, social cues from the external environment initiate a specific signaling pathway in the mother that leads to the modulation of non-coding RNA in the progeny. To identify a throughline from environmental social cues to modulation of embryonic gene silencing, I have begun to interrogate different aspects of this maternal signaling pathway: the neurocircuitry, key molecular components, and tissue specificity. As this maternal signaling can respond to environmental cues, this work lends to a model where the maternal environment can modulate embryonic gene expression and impact development. Ultimately, this work will lead to a clearer understanding of the mechanisms involved in cross-generational signaling between mother and progeny.

807A **Structure-function analysis suggests that the photosensor LITE-1 is a light-activated ion channel** Sonya Hanson[1], Jan Scholuke[2], Jana Liewald[2], Rachita Sharma[2,3], Gerhard Hummer[3], Alexander Gottschalk[21]Flatiron Institute, [2]Goethe University, [3]Max Planck Institute of Biophysics

C. elegans detects UV and blue light via the photosensor proteins LITE-1 and GUR-3 [1,2]. Previous work showed that LITE-1 absorbs UV photons with an unusually high extinction coefficient, involving essential tryptophans [3]. Yet, how this UV light sensation is translated into a cellular response remains elusive, and also, how tryptophans account for the behavioral sensitivity to blue light is unclear. We modeled the structure LITE-1 using AlphaFold2(AF2)-multimer and molecular dynamics (MD) simulations, and performed mutational and behavioral assays to probe the model. The LITE-1 structure resembles ion channels, and MD simulations predict formation of a stable, closed channel. Based on the model, we identified likely channel lining residues. To test their functional importance, LITE-1 and mutant variants were expressed in muscle cells, where blue light exposure induces pronounced contraction. Mutation of putative channel-gating residues abolished function in this assay. Efforts to demonstrate light-gated channel function in dissected muscle or in *Xenopus oocytes* by electrophysiology were inconclusive, indicating that dissection conditions negatively affect channel gating, and/or that in oocytes an unknown cofactor may be absent. Other mutations identified in genetic screens affect amino acid positions that suggest important roles in protein stability or multimer assembly. We identified a binding pocket that may either accommodate a putative chromophore, or a photo-oxidation product generated in tissue upon UV absorption. Several of the residues lining this pocket were previously established as essential for LITE-1 function. A newly-identified critical cysteine pointing into the pocket represents a likely attachment site. We derived a model for how photon absorption, via a network of tryptophans and other aromatic residues, induces an excited state that is transferred to the bound molecule, to evoke protein conformational changes leading to channel gating. GUR-3, which is required for UV-responses in the pharynx, may use a similar mechanism for photon detection, as suggested by its AF2 model. Thus, a common protein fold and likely ligand-gated assembly, possibly by binding of a particular compound, appears to have evolved into a light-activated ion channel.

1. Edwards et al. (2008) PLoS Biol 6, 0060198
2. Bhatla and Horvitz (2015) Neuron 85, 804-18
3. Gong et al. (2016) Cell 167, 1252-63

808B **WormPicker: A general-purpose automated system for high-throughput genetic manipulation and analysis of *C. elegans*** Zihao Li[1], Anthony Fouad[1], David M Raizen[2], Chris Fang-Yen[31]Bioengineering, University of Pennsylvania, [2]Neurology, University of Pennsylvania, [3]Biomedical Engineering, Ohio State University

Automated techniques for genetic manipulation of *C. elegans* have the potential to improve productivity compared to manual methods, especially when working with a large number of strains. Previous automated methods based on microfluidic devices and/or flow cells are limited in their repertoire, especially for genetic manipulation. Here, we describe WormPicker, a robotic system capable of performing complex genetic procedures by autonomously imaging and picking *C. elegans*. WormPicker uses a 3D motorized stage to move an imaging system and a robotic picking arm over an array of up to 144 standard agar plates. Machine vision algorithms track individual animals in both low and high resolution, and assay phenotypes including developmental stage, sex, morphology, and expression of fluorescent reporters. According to these phenotypes, the robotic arm selectively picks individual worms using an electrically self-sterilizable platinum wire loop, guided by machine vision and electrical capacitive touch sensing. For a fluorescent animal sorting task, WormPicker picked worms at a speed of about 3 animals per minute, a throughput comparable to that of a group of researchers having a median working experience of 5 years. We developed multi-purpose scripting toolsets for the system to perform complex, arbitrary genetic manipulations. We validated WormPicker's effectiveness and versatility by automating a collection of common *C. elegans* genetic procedures, including genetic crossing, genetic mapping, and integrating extrachromosomal arrays. We are using our system to perform an automated screen for genes that modulate stress-induced sleep using the Million Mutation Project strain library. Our system will accelerate a broad range of *C. elegans* research and open doors to genetic and pharmacological screens that would be impractical by conventional approaches.

809B **Loss of *hlh-3* causes an interneuron to take on a sensory neuron-like morphology** Berenice Chavez Rojas[1,2], Meaghan K. Carey[2,3], Elizabeth R. Cebul[2,3], Maxwell G. Heiman[2,31]Genetics, Boston Children's Hospital, [2]Harvard Medical School, [3]Boston Children's Hospital

During vertebrate development, neurons arise from neuroepithelia through a process called "delamination," which involves the down-regulation of apical junctions and detachment from the epithelium. Many steps in this process remain unclear, but it is thought to be regulated primarily by bHLH transcription factors. To better understand delamination, we focused on the development of the C. elegans amphid sense organ, which consists of 12 sensory neurons and two glial cells that are organized as a tube-shaped epithelium. In the early stages of development, the amphid neurons and glia form a cellular rosette, in which the cells are polarized towards an apical vertex. As the embryo develops, the vertex of the rosette attaches to the anteriorly migrating epidermis and is dragged toward the nose tip. Amphid neuron cell bodies then move away from the nose, but their dendrites remain anchored at the nose tip through epithelial apical junctions and are stretched out behind them in a process called retrograde extension. Importantly, the rosette also includes several non-amphid neurons that, in the mature animal, project axons into the nerve ring but do not have sensory dendrites extending to the nose. Thus, these non-amphid neurons presumably disengage from the amphid epithelium at a certain point during this process. The molecular mechanisms that signal disengagement have not been investigated, and we hypothesized that they might be similar to delamination in vertebrates. We focused on one of the non-amphid rosette neuron, RIV, which extends an axon that enters the nerve ring subdorsally and runs around the nerve ring to create a loop-like shape. We reasoned that, if RIV failed to disengage from the rosette, it might remain attached at the nose and thus develop an anterior neurite like the amphid neurons. Through a forward genetic screen, we isolated a mutant in which the RIV cell body is misplaced and the neurite fails to form a loop, instead extending towards the nose tip in a morphology that resembles that of an amphid sensory neuron. We identified the bHLH transcription factor hlh-3 as the causative gene in this mutant, suggesting that expression of hlh-3 may be required for non-amphid neurons to disengage from the amphid epithelium similar to the role of bHLH transcription factors in vertebrate neurodevelopment. Together, our results point to a possible conserved mechanism controlling the differentiation of neurons that delaminate from neuroepithelia.

810B **A Neural Circuit for Proprioceptive Control of Undulatory Movement in *C. elegans*** Hongfei Ji[1], Anthony Fouad[2], Zihao Li[2], Andrew Ruba[2], Chris Fang-Yen[11]Biomedical Engineering, Ohio State University, [2]Bioengineering, University of Pennsylvania

Proprioception is crucial for motor behavior, providing feedback as an animal adapts to its immediate physical environment. Here, we characterize the proprioception-mediated homeostatic control of undulatory movement in *C. elegans*. We found that the worm responds to optogenetically or mechanically induced decreases in mid-body bending amplitude by increasing its anterior amplitude and conversely responds to increases in mid-body bending amplitude by decreasing its anterior amplitude. Using genetics, microfluidic and optogenetic perturbation response analyses, and optical neurophysiology, we elucidated the neural circuit underlying this compensatory motor response. The dopaminergic PDE neurons proprioceptively react to mid-body

bending and signal to AVK interneurons via the D2-like dopamine receptor DOP-3. The FMRFamide-like neuropeptide FLP-1, released by AVK, regulates SMB head motor neurons to modulate anterior bending. We propose that this homeostatic behavioral control optimizes locomotion efficiency. Our findings provide a mechanism in which proprioception works with dopamine and neuropeptide signaling to mediate motor control.

811B **Systematic overexpression screen of human 21 Chromosome genes in *C. elegans*** Sophia Sanchez[1], Brooke Frohock[2], Briana Syed[2], Eva Beckett[2], James Groh[2], Sofia Smith[2], Katherine Perks[2], Ella Demott[2], Reece Jones[2], Nina Mourao[2], Ansley Fiorito[2], Jon Pierce[21]Neuroscience, University of Texas at Austin, [2]University of Texas at Austin

Down syndrome is the most common genetic cause of intellectual disability. Although we know that trisomy 21 causes Down syndrome, we still do not know which of the close to 200 HSA21 genes cause Down syndrome phenotypes. While mouse models have been used to identify causal roles for a handful of HSA21 genes in Down syndrome, the vast majority of HSA21 genes have not been tested. With the genetically tractable model organism Caenorhabditis elegans, we are systematically testing all HSA21 orthologs for overexpression phenotypes. C. elegans shares orthologs (51 of which are highly conserved) for over half of the protein-coding HSA21 genes excluding those that encode keratin. We are generating transgenic strains that overexpress each of the highly-conserved orthologs. Next, we are using high-throughput, quantitative behavioral assays to deduce which genes cause developmental abnormalities and/or neuromuscular defects when overexpressed. To date, we have discovered several HSA21 orthologs that cause locomotion defects when overexpressed, suggestive of neural or muscular deficits, including orthologs of CHAF1B, DONSON, EVA1C, and PFKL. We are now revealing the mechanistic bases for these behavioral defects, and find, for example, that overexpression of the EVA1C ortholog causes partially-penetrant defects in axon guidance and cell migration. We also find that overexpression of many Hsa21 orthologs causes severe, but lowly-penetrant defects, which could help explain the incomplete penetrance of numerous comorbidities in DS. By determining which HSA21 orthologs cause behavioral and cellular phenotypes when overexpressed in C. elegans, we hope to identify novel therapeutic targets and treatments for people with Down syndrome.

812B **Natural variants in orthologs of autism and Williams syndrome risk genes account for variance in social clumping in *Caenorhabditis elegans*** Brooke Frohock[1], Kaelin Rubenzer[1], Maria Noonan[1], Courtney Williams[1], Emily Ricketson[1], Zheng Wu[2], Erik Andersen[3], Jonathan Pierce[11]Neuroscience, University of Texas at Austin, [2]University of Texas at Austin, [3]Northwestern University

Human studies have identified over 100 genes that are confidently associated with autism, a spectrum disorder characterized by atypical social behaviors. In parallel, animal studies have found that natural variants in orthologs of a few of these genes are associated with the degree of social behaviors displayed by social species such as bees, ants, and prairie voles. The nematode *Caenorhabditis elegans* aggregates into clumps in part using pheromones. We sought to determine if natural variants in orthologs of these high-confidence ASD risk genes were associated with social clumping in *C. elegans*. We found that whereas many wild strains from around the globe are highly social, the number of ASD risk orthologs with high impact variants correlated with lower levels of clumping. Investigating variants in specific genes, we found that the most deleterious variants correlated with low clumping. Further analysis of variants across genes found that those in genes that correlated negatively with clumping were associated with severe ASD. Variants in some genes (SYNGAP1/*gap-2*, NLGN/*nlg-1*, and DYRK1A/*mbk-1*) appear to cause low clumping because transformation of wild strains with reference versions of these genes boosted clumping. Other genes that correlated positively with clumping have human versions that are either associated with incompletely penetrant ASD (e.g. TBR1/*tbx-8*), or are candidate modifiers of high sociality in ASD (IL-17R/*ilcr-1*) or Williams syndrome (DMPK/*mrck-1*). Variants in these three genes, as well as the Williams syndrome (WS) critical region gene, BAZ1B/*athp-2*, appear to cause high clumping because mutation of these orthologs boosted clumping in wild and lab strains. Our results suggest that many orthologs relevant to ASD or WS have roles in modifying natural variation in *C. elegans* social behavior. Further study of these genes in *C. elegans* may reveal mechanisms regarding their function and dysfunction leading to insight regarding these neurodevelopmental disorders.

813B ***C. elegans* males optimize mate-choice decisions via sex-specific responses to multimodal sensory cues** Jintao Luo[1], Arantza Barrios[2], Douglas S Portman[31]Xiamen University, [2]University College London, [3]Dept of Biomedical Genetics, Univ Rochester Sch Med Dent

For sexually reproducing animals, selecting optimal mates is essential for maximizing reproductive fitness. Because *C. elegans* reproduces primarily by self-fertilization, little is known about its mate-choice behaviors. While several classes of sensory cues have been implicated in males' ability to recognize hermaphrodites, achieving an integrated understanding of how males use these cues to determine the stage and sex of potential mates, particularly when they are presented in their native context, has proven challenging. Here, we use a choice-based social-interaction assay to explore the ability of *C. elegans* males to make and optimize their mate choices. We find that males use a combination of volatile sex pheromones (VSPs), ascaroside pheromones, surface-bound chemical cues, and other signals to assess a variety of features of potential mates. Each of these

signals is important for communicating specific aspects of mate choice: VSPs likely signal the presence of a sperm-depleted, physiologically female hermaphrodite; ascaroside cues help males assess the sex and stage of potential mates as well as their nutritional status; and ascaroside and surface-associated cues cause males to prefer virgin over cross-fertilized hermaphrodites. The male-specificity of these behavioral responses stems from both male-specific neurons and the male state of sex-shared circuits, and we reveal a previously undescribed role for the sex-shared ASH amphid sensory neurons in male attraction to endogenously produced hermaphrodite ascarosides. Together, our findings lead to a multistep, flexible view of the behavioral sequence by which males use diverse sensory cues to assess multiple features of potential mates and optimize mate choice.

814B **Transcriptional profiling and clearance of *apl-1* and its gene product APL-1, the C. elegans orthologue of human APP** Chris Li[1,2], Ji-Sup Yang[3], Alessandro Mercado[1], Jessica Zavalunova[1], Jaymie Paredes[11]Biology, City College of New York-CUNY, [2]Graduate Center, CUNY, [3]Biology, Graduate Center-CUNY

Alzheimer's disease (AD) is a neurodegenerative disease that affects over 50 million people worldwide. The brains of AD patients are characterized by the presence of neurofibrillary tangles, whose major component is phosphorylated tau, and amyloid plaques, whose major component is the beta-amyloid peptide (Abeta). Abeta is a cleavage product of the amyloid precursor protein (APP), which is part of the APP family in mammals. Loss of the mammalian APP family results in lethality. Similarly, loss of APL-1, the *C. elegans* APP orthologue, also leads to lethality. Like mammalian APP, APL-1 is cleaved to release an extracellular fragment, sAPL-1, and an intracellular fragment, AICD. sAPL-1 is necessary and sufficient for viability. High levels of APL-1 in transgenic animals also lead to lethality.

Because high levels of APL-1 lead to lethality, we examined how excess sAPL-1 is cleared. Does excess sAPL-1 get scavenged by glial cells, the equivalent of mammalian astrocytes, or by coelomocytes, the equivalent of mammalian microglia? By tagging the extracellular and intracellular domains of APL-1 with sfGFP and wrmScarlet, respectively, we determined that sAPL-1 is scavenged by coelomocytes and not by glial cells. These data suggest that in mammals, microglia are responsible for clearing not only Abeta, but also excess sAPP.

To identify genes whose expression patterns are altered with changes in APL-1 levels, we are currently analyzing the transcriptomes of *apl-1* mutant and transgenic lines . Genes that are up- or down-regulated in the *apl-1* disrupted strains will be cross-referenced to genes found to be differentially regulated in mouse APP knockouts. Overlapping genes will be further examined for interactions with APL-1.

815B **GOA-1 affects different parameters of mechanosensory habituation** Alvaro Luna[1], Aaron Reiss[2], Catharine Rankin-[11]Psychology, University of British Columbia, [2]University of British Columbia

Heterotrimeric G proteins are membrane-associated G proteins that are involved in signaling pathways from the cellular membrane to intracellular substrates like the pre-and postsynaptic membranes of neurons. The Ga_o subunit is the most abundant heterotrimeric G protein in the brain. Physiologically, the Ga_o signaling pathway is associated with the depression of neural activity. The *Caenorhabditis elegans* ortholog of Ga_o, GOA-1, is >80% identical to its mammalian counterpart by nucleic acid sequence and is expressed in virtually all neurons in the worm. Previous research suggests that GOA-1 plays a regulatory role in habituation of response probability and duration on mechanosensory stimuli in *C. elegans*. Habituation is a form of non-associative learning defined as the decrease in responding to repeated stimulation, which cannot be attributed to sensory adaptation or motor fatigue. This form of basic learning is hypothesized to allow animals to identify and ignore irrelevant stimuli. In this study, we investigated the role of GOA-1 for habituation using the Multi-Worm Tracker (MWT), a high-throughput machine that simultaneously quantifies the behaviour of many *C. elegans,* and determining where *goa-1* is acting by using cell-specific targeted degradation of the GOA-1 protein and testing behaviour. We employed the GFP-nanobody ZIF-1 degron approach to engineer strains for tissue-specific degradation of GOA-1. First, we generated a GFP CRISPR knock-in strain into the endogenous GOA-1 locus within an internal loop of the final polypeptide structure. Then, we expressed GFP nanobody::ZIF-1 driven by different promoters to specifically degrade GFP-tagged GOA-1 proteins in touch receptor neurons (Pmec-18), chemosensory neurons (Posm-6) and pan-neuronal degradation (Prgef-1). The insertion of GFP seems to alter behaviour in unexpected ways. It affected habituation of response probability and not response duration. We also compared the CRISPR goa-1::GFP strain with a MosSCI single copy insertion of GFP in the same location in a goa-1(sa734) mutant and we had the same results. Finally, we found that tissue-specific degradations of GOA-1 had different effects on habituation of reversal probability and duration habituation with no conclusive results.

816B **A community framework for development of of C. elegans whole-brain imaging analysis pipelines** Daniel Y Sprague[1], Jackson Borchardt[1], Raymond Dunn[1], Kevin Rusch[2], Grace Chiu[1], Eviatar Yemini[2], Saul Kato[11]Neurology, University of California, San Francisco, [2]Neurobiology, University of Massachusetts, Chan Medical School

Since the advent of whole-brain imaging techniques in C. elegans ten years ago, there has been a major need for an effective

and efficient data analysis pipeline to go from raw volumetric microscopy videos to identity-labeled neural activity time series. A common approach is to decompose this pipeline into three steps: segmentation, identification, and quantification. Many algorithms and tools attacking these challenges have been released, but a lack of accuracy, completeness, robustness, and automation for all of these analytical steps remains, impeding the widespread adoption of whole-brain imaging. Leveraging advances in machine vision, strides have been made in automated algorithmic approaches, but these approaches still require significant manual annotation, verification and correction; therefore interactive visualization and curation tools are required to produce high-quality labeled time series datasets. Recently, polychromatic neuron-tagged C. elegans strains, such as NeuroPAL, have been developed, which substantially aid the identification of neuron identities, and work is ongoing to leverage this technology in automated analysis pipelines.

We present a community framework for development of C. elegans whole brain imaging analysis pipelines, consisting of the following components: a common data format for C. elegans whole-brain imaging datasets, extending the popular Neurodata Without Borders (NWB) format; a set of pre-processing tools to enable the incorporation of diverse datasets for algorithm training; a curated online repository for reference datasets contributed by various labs; reference open-source Python and MATLAB analysis pipelines and 4D data exploration GUIs built on the Chan Zuckerberg Initiative's multi-dimensional image viewer napari and our own NeuroPAL ID software; and finally a set of visualization tools for exploring and benchmarking algorithmic results. We hope these resources will catalyze the improvement of crucial analytical tools and open up the use of these computationally intensive experimental approaches to the field.

817B **Investigating the roles of somatostatin-like receptors npr-16 and npr-24 on C. elegans behaviours.** William Bendena, Sanaz Biglou, Jeff Boudreau, Foroozan Torki, Dan Quesnelle, Ian Chin-SangBiology, Queen›s University

The conserved neuropeptide signaling pathway in *C. elegans* provides a promising opportunity to further advance our knowledge regarding the molecular basis of behaviour and physiology in higher organisms while paving the way for potential development of therapies for various neurodegenerative disorders. In mammals, the peptide hormone family somatostatin comprises a group of various secretory proteins that control neurotransmission, metabolism, and memory by acting on their respective G-Protein Coupled Receptor (GPCR) to inhibit downstream growth-related genes. In *C. elegans* over 50 GPCRs act as neuropeptide receptors (*npr*), many of which have mammalian orthologs including *npr-16* and *npr-2*4. Thus, characterization of these receptors will allow discovery of analogous somatostatin signaling in *C. elegans* that can be used for studying the impact of specific neuropeptide and hormonal dysregulation in humans. Through phenotypic and genetic analysis, we have examined the role of *npr-16* and *npr-2*4 in *C. elegans* and determined the potential pathways they operate in by establishing an evolutionary link across species. Our work has determined the impact of knock out mutations on key growth phenotypes in of the *C. elegans*, including longevity and vitellogenesis, as well as their role in metabolism as shown through the distinct increase in fat content. Furthermore, we have observed a potential evolutionary link between *C. elegans npr-16* and *npr-24* and *Drosophila* allatostatin-C by examining the impact of the mutation on key enzymes involved in Juvenile hormone biosynthesis. Characterization of *npr-16* and *npr-24* will provide a unique opportunity to enhance our knowledge about growth and development in *C. elegans* and aid further somatostatin-related therapeutic studies in this model organism.

818B **Investigating behavioral responses to fire-exposed plant material in *C. elegans*** Kanaili Singkeo[1], Jahna Thompson[2], Charles Fisher[2], Arielle Halpern[2], Melissa LaBonty[31]Southern Oregon University, [2]Southern Oregon University, [3]Biology, Southern Oregon University

The American west coast has a rich history with wildfire as a natural mechanism for maintaining healthy forest ecosystems. However, with the recent effects of climate change, wildfires in this region have increased in frequency and intensity. This increase in wildfire severity highlights an urgent need for research into the impacts of wildfire exposure on important regional food sources. We hypothesize that *C. elegans* can be used as a tool to investigate whether plant-based food sources exposed to wildfires undergo changes in chemical composition that impact animal behaviors, such as foraging and food preference. We also suspect that there are species in wildfire-prone regions that may have adapted over time to the impacts of fire and smoke on plant material, developing tolerance or even preference for these modified food sources. In our experiments, we first investigated whether *C. elegans* can detect changes in the chemical composition of plants as a result of wildfire exposure. We used chemotaxis assays for attraction and repulsion to quantify behavioral responses in wildtype *C .elegans* to extracts derived from acorns of the Oregon white oak (*Quercus garryana*). The acorn extracts were prepared from material collected from white oak unaffected by wildfire and from trees exposed to the Almeda wildfire that impacted Southern Oregon in Fall 2020. We also assessed how regionally-isolated nematodes compared to wildtype *C. elegans* in their responses to fire-exposed acorn extracts. We collected nematodes from the Rogue Valley in Southern Oregon and conducted attraction and repulsion assays with the same acorn extracts described above. We found that wildtype *C. elegans* show slight attraction to extracts from unexposed acorns, but exhibit repulsive behaviors in response to fire-exposed acorn extracts. In contrast, locally-isolated nematodes showed tolerance

rather than repulsion to fire-exposed acorn extracts. These results suggest that fire exposure causes an impact on the chemical composition of acorn extracts that is detectable by *C. elegans* and that local nematode isolates may have evolved to exhibit tolerance for fire-exposed plant material. Future experiments will use analytical methods to determine the key compounds in acorn extracts impacting *C. elegans* behavior, with the goal of investigating how these key compounds might play a larger role in foraging and food preferences in a variety of species found in wildfire-prone regions.

819B **Transcriptomic changes in *C. elegans* with mixed tau and TDP-43 pathology** Vaishnavi Jadhav[1], Randall Eck[1], Caitlin Latimer[1], Brian Kraemer[1,2], Nicole F. Liachko[1,3]1University of Washington, [2]VA Puget Sound Health Care System, [3]GRECC, VA Puget Sound Health Care System

Alzheimer's disease (AD), the most common aging-associated neurodegenerative dementia disorder, is defined by the presence of amyloid beta (Aβ) and tau aggregates in the brain. However, more than half of patients also exhibit aggregates of the protein TDP-43 as a secondary pathology. Clinically, AD patients with secondary TDP-43 pathology have more severe cognitive impairment, more rapid cognitive decline, worse brain atrophy, and a shorter disease course. TDP-43 is already implicated in neurodegenerative disease as the major pathological protein aggregate in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP), two other devastating neurodegenerative diseases. In patients with mixed Aβ, tau and TDP-43 pathology, TDP-43 dysfunction may synergize with neurodegenerative processes in AD, worsening disease. We have developed *C. elegans* models of mixed pathology in AD by co-expressing human Aβ, tau and TDP-43 pan-neuronally. Using these models, we have found that TDP-43 specifically synergizes with tau but not Aβ, resulting in enhanced neuronal dysfunction, selective neurodegeneration, and increased accumulation of pathological tau. To identify cellular responses to mixed tau and TDP-43, we are evaluating transcriptomic changes at multiple time-points preceding frank neuronal loss in *C. elegans*, and assessing similarities and differences in gene expression from human AD brain with co-pathological TDP-43. We find significant expression and splicing changes in genes including those implicated in immune function, RNA metabolism, synaptic integrity, and lipid catabolism. Characterizing transcriptomic changes resulting from mixed tau and TDP-43 pathology and determining their underlying contributions to disease processes is critical for understanding mixed pathology AD.

820B **Pheromone-based animal communication influences the production of somatic extracellular vesicles** Katarzyna Banasiak[1], Agata Szczepańska[2], Klaudia Kołodziejska[2], Abdulrahman Ibrahim Tudu[2], Wojciech Pokrzywa[1], Michał Turek[2,1]Laboratory of Protein Metabolism, International Institute of Molecular and Cell Biology in Warsaw, [2]Laboratory of Animal Molecular Physiology, Institute of Biochemistry and Biophysics Polish Academy of Sciences

Extracellular vesicles (EVs) are involved in multiple biological processes. To date, most studies have focused on the intracellular molecular mechanisms of EVs biogenesis and consequently, there is limited knowledge of the influence of environmental factors or other individuals in the population on the activity of EV-regulated systems at the organismal level. We have previously shown that the largest evolutionarily conserved EVs, exophers, are a component of the *C. elegans* maternal somatic tissue resource management system induced by the embryos developing *in utero*. As a result, the progeny of individuals with active exopher biogenesis (exophergenesis) appear to be better adapted to thrive in the habitat. Using this model, we investigated the inter-tissue and social regulatory mechanisms of exophergenesis. We found that exophergenesis activity is differentially modulated by sex-specific ascaroside (pheromones) signaling molecules, known to have multiple functions in development and behavior. While hermaphrodite-released pheromones down-regulate exophergenesis, male-released pheromones favor strong exopher production. This ascaroside-dependent regulation is fine-tuned by exopher-promoting olfactory neurons exposed to the environment (partially via STR-173 seven TM receptor) and exopher-inhibiting sensory neurons exposed to the body cavity. Together, we uncovered critical control nodes for somatic EVs production mediated by the nervous system in response to social cues.

821B **Combined TDP-43 and tau pathology leads to selective neurotoxicity in *C. elegans*** Caitlin S. Latimer[1], Jade G. Stair[2], Joshua C. Hincks[2], Heather N. Currey[2], C. Dirk Keene[1], Brian C. Kraemer[1,2,3,4], Nicole F. Liachko[4,5]1Laboratory Medicine and Pathology, University of Washington, [2]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, [3]Psychiatry and Behavioral Sciences, Unviersity of Washington, [4]Medicine, University of Washington, [5]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle

Age-related cognitive decline is often attributed to Alzheimer's disease (AD) pathology, which is defined by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau. The exact mechanisms by which these protein aggregates lead to neurodegeneration are unknown, but tau correlates better with cognitive impairment than amyloid pathology and is likely an important contributor to neuronal dysfunction and loss. Further, it is widely accepted that there are selective vulnerabilities of specific neuron subtypes to tau pathology. While AD is the most common neurodegenerative disease of aging, aggregates of a third protein, TDP-43, have been identified as a common co-pathology. Data from human studies suggest that synergistic interactions between tau and TDP-43 may lead to worsened disease outcomes, including more severe and rapid cognitive decline during life and higher tau burden at autopsy. However, the mechanisms that underly this ob-

served synergy are unknown, including whether there are neuronal subtypes that are selectively vulnerable to this co-pathology.

We have developed new models of tau and TDP-43 co-pathology using *C. elegans* and have previously shown that TDP-43 specifically enhances both total and pathologic tau burden. These animals also have more severe motility deficits than those expressing either protein alone. Interestingly, this synergism between tau and TDP-43 is rescued by loss-of-function of the robust tau modifier *sut-2,* implicating enhanced tau neurotoxicity as the primary driver underlying the worsened clinical and neuropathological phenotypes in AD with TDP-43 pathology. To further characterize this model, we have performed assays of neurodegeneration. Specially we used GFP reporters specific for distinct neurotransmitter classes of neurons (dopaminergic, glutamatergic, serotonergic, cholinergic or GABA-ergic) and measured the degree of neuron loss. We also evaluated the effect of aging by assessing neurodegeneration at day 1 and day 4 of adulthood. Our results reveal distinct differences in neuron subtype vulnerabilities to co-morbid tau and TDP-43, and to aging. Uncovering the mechanisms that underlie these cell-type specific sensitivities to tau alone versus TDP-43 and tau is crucial to understand, and ultimately treat, mixed pathology AD.

822B **PPRP-1/PHACTR1 holophosphatase controls SV cycle in C. elegans** patrick laurent[1], Miguel Soler Garcia[1], Katerina Stratigi[1], Mia Krout[2,3], Janet Richmond[21]UNI, Université Libre de Bruxelles, [2]Department of Biological Sciences, University of Illinois at Chicago, [3]Dept. of Biological Sciences, University of Illinois at Chicago

The activity-dependent fusion, retrieval and recycling of synaptic vesicles (SV) is essential for neurotransmission. A forward genetic screen to identify genes involved in neuromodulation by neuropeptides we isolated *snn-1*/synapsin and *pprp-1*/PHACTRs. Synapsins are key phosphoproteins involved in SV recycling and SV clustering, they are associated to seizure in mouse and human. PHACTRs form holophosphatases together with Protein Phosphatase 1 (PP1). The formation of these holophosphatase is regulated by actin dynamics. PHACTR1 autosomal dominant variants are associated to Developmental Epilepsy and Encephalopathy 70 (DEE70). Using *C. elegans*, we dissected *pprp-1*/PHACTRs structure and its neuronal functions in-vivo. Mimicking DEE70 mutations in *pprp-1* gene generated constitutively active holophosphatase by reducing its inhibition by G-actin. The holophosphatase reduced neurotransmission at neuromuscular junction by a mechanism that include the control of Synapsin phosphorylation: Serine 9-Synapsin is dephosphorylated in *pprp-1(DEE70)* while it is hyperphosphorylated and spread in the axon in *pprp-1(null). pprp-1(DEE70)* modify the Synaptic Vesicle (SV) cycle: reuptake of SV membrane protein by local endocytosis is reduced, faster synaptic fatigue was observed that likely corresponds to exhaustion of SV recycling as we observed a reduced number of SV at synapses and abnormal endocytic patterns by EM of the NMJ. In our model, G-actin depletion occurring in synaptic bouton is used as a signal for holophosphatase formation and dephosphorylation of substrates important for SV recycling or clustering. DEE70 variants would constitutively activate this presynaptic mechanism.

823B **Shaping Brain Function with Microbes** Elizabeth DiLoreto, Jagan SrinivasanBiology and Biotechnology, Worcester Polytechnic Institute

Animal behavior is shaped by neuropeptides. These small neuromodulators regulate synaptic communication in the nervous, immune, and endocrine systems by changing the response of G-protein coupled receptors. Neuropeptides flexibly repurpose neural circuits when a single gene encodes multiple peptidergic regulatory elements. In humans, there are over 100 neuropeptide genes that make hundreds of individual neuropeptides, with more still being classified. In *C. elegans*, we have more genetic transparency and there have been upwards of 350 neuropeptides identified in three functional classes that are post-translationally modified from 131 neuropeptide genes. The ability of a gene to encode multiple peptides makes it difficult to identify roles of discrete and active neuropeptides since a single neuropeptide gene can make multiple active peptides. We have developed a technique to study the function of neuropeptides by encoding a single neuropeptide gene in our patented vector design, introducing it into an *E. coli* vehicle, and feeding it to our model system *C. elegans.* Here, we present this novel tool as a method to functionally rescue neuropeptides in genetic loss-of-function *C. elegans* mutants. Our neuropeptide rescue-by-feeding system permits the study of neuropeptides at single peptide resolution, in a simple, effective system. Since the peptides enter the worm via its food source, this method opens avenues to further investigate the connection between the brain and gut. There are also potential therapeutic applications with the ability to bolster the gut microbiome to support brain function, by releasing the target neuropeptide to shape neural circuit function and behavior. We are currently working to determine the mechanism by which our peptide delivery functions and to further optimize this application.

824B **Interaction Rules behind emergence of *C. elegans* aggregation** Youn Jae Kang[1], Serena Ding[21]Max Planck Institute of Animal Behaviour, [2]Max Planck Institute of Animal Behvaiour

Caenorhabditis elegans aggregation is commonly observed under certain laboratory conditions, yet little is known about why it occurs. While the precise function of aggregation remains elusive, here I ask whether aggregation can simply be understood as an emergent phenomenon resulting from simple individual interaction rules, some of which may be due to biological drivers while others are simply the result of mechanical properties.

Here I report new observations supporting two potential interaction rules behind worm aggregation: collision between worms leading to alignment, and speed changes that correlate with local oxygen level.

Group of worms with individual heads tagged with GFP are tracked on an agar plate. Worms from the aggregating strain *npr-1(ad609)* aligned their head directions as they formed an aggregate, unlike those from the N2 reference strain which did not cluster and therefore showed nearly random distributions of head orientations in the same behaviour environment. Mechanical collision results in the alignment of the direction of velocity between the objects that collided, which suggests that this may be an important factor in forming aggregates.

As for the speed change, I use a real-time oxygen visualisation technique to show that worm speed decrease tightly correlates with low local oxygen levels in space and time. I observed that local oxygen depletion was largely due to consumption by the worms instead of by the bacteria on which they feed, given that the concentration of bacteria was not too high. Previous report with ambient oxygen has shown that decreasing oxygen level makes worm's speed drop in real time (Zimmer et al., 2009, Neuron). Also, the speed difference in low oxygen condition was much more significant in *npr-1* mutants than N2 (Rogers et al., 2006, Current Biol). Taking these information into account, in areas of low-oxygen, *npr-1* worms decelerate much more than N2, suggesting that this speed change may be another potential driver of aggregation.

To test how these independent rules can act in conjunction, I will assess if aggregation emerges in an agent-based simulation, where each agent follows the two interaction rules. This work could show how simple and independent rules can give rise to unexpected complexity, with *C. elegans* aggregation potentially being one such phenomenon.

825B **Pheromone perception during early development remodels neurodevelopment and accelerates neurodegeneration in adult *C. elegans*** Jingyi Peng[1], Xuqing Liu[1], Xian-Ting Zeng[1], Yue Hao[1], Jia-Hui Zhang[1], Qian Li[2], Xia-Jing Tong[11]School of Life Science and Technology, ShanghaiTech University, [2]Shanghai Jiao Tong University School of Medicine

The aggregation of misfolded proteins is a pivotal factor in causing age-associated neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. The etiology of these neurodegenerative diseases shares environmental components, including chemical cues. However, how chemical cues modulate neurodegeneration remains unclear. Here we found that in *Caenorhabditis elegans*, exposure to pheromones (containing ascr#3 and ascr#10) in the very early developmental stage (L1 stage) remodels neurodevelopment and accelerates α-synuclein-induced neurodegeneration without affecting lifespan. Perception of ascr#3 and ascr#10 is mediated by two pairs of chemosensory neurons, ASK and ASI, respectively. Activation of both ASI and ASK neurons in the L1 stage is required and sufficient to remodel neurodevelopment via AIA interneurons, which triggers insulin-like signaling in adult neurons in a non-cell-autonomous manner. We further show that distinct signaling pathways mediate the effects of ascr#3 and ascr#10. ascr#3 is perceived by GPCR DAF-38 in ASK neurons and activates glutamatergic transmission into AIA interneurons. ascr#10 is perceived by GPCR STR-2 in ASI neurons and activates the secretion of neuropeptide NLP-1, which then binds to the NPR-11 receptor in AIA interneurons. Our work reveals a mechanism for how pheromone perception at the early developmental stage modulates proteotoxicity-induced neurodegeneration in adults, and provides insights into how the external environment impacts the progression of neurodegenerative diseases.

826B **Investigating mechanisms of dendritic pruning in *Caenorhabditis elegans*** Paola Figueroa-Delgado[1], Shaul Yogev[21]Department of Cell Biology, Yale University, [2]Department of Neuroscience, Yale University

Neurite remodeling is a highly conserved process that refines and establishes a mature nervous system. A failure in neurite remodeling leads to neurological and neurodevelopmental disorders. While developmental dendritic pruning, a means of neurite remodeling, has been extensively studied, the cell-biological mechanisms that control pruning remain poorly understood. The nematode *Caenorhabditis elegans* inner labial 2 (IL2) neurons, upon entering a developmental diapause, extend a stereotypical dendritic arbor that is pruned when reproductive development is resumed – leaving primary dendrites intact. The stereotypic remodeling of IL2 neurons allows experimental access to elucidate the cell-biological mechanisms that underlie pruning. To identify IL2 pruning regulators, I conducted an unbiased forward genetic screen to identify pruning defective mutants. I isolated a novel mutant allele of *sax-1*, a gene encoding a serine/threonine kinase, which yields a severe loss-of-pruning defect characterized by the remanence of the dendritic arbor into adulthood post-dauer exit. SAX-1 is the *C. elegans* homolog of mammalian nuclear dbf2-related (NDR) kinases, which are conserved from yeast to humans and have been shown to regulate neuronal outgrowth, termination, and tiling in *Drosophila* and *C. elegans*. I show through cell-specific rescue experiments that SAX-1 acts cell-autonomously to modulate IL2 higher-order dendrite pruning in a kinase activity dependent manner. To address if SAX-1 acts with known conserved scaffolding and interacting protein, SAX-2, I tested a loss-of-function allele for *sax-2*, which phenocopied *sax-1* mutants post-dauer exit. These findings suggest that SAX-1 and SAX-2 work together to regulate IL2 neuronal remodeling. Identifying how SAX-1 mediates IL2 higher-order dendrite pruning post-dauer exit will provide mechanistic insights into the cell-biological regulation of neuronal remodeling.

827B **UNC-43/CaMKII-triggered anterograde signals recruit $GABA_A$Rs to mediate inhibitory synaptic transmission and plasticity at *C. elegans* NMJs.** Yue Hao[1], Haowen Liu[2], Xian-Ting Zeng[1], Ya Wang[3], Wan-Xin Zeng[1], Kang-Ying Qian[1], Ming-Xuan Chi[1], Shangbang Gao[3], Zhitao Hu[2], Xia-Jing Tong[11]School of Life Science and Technology, ShanghaiTech University, [2]Queensland Brain Institute, Clem Jones Centre for Ageing Dementia Research (CJCADR), The University of Queensland, [3]College of Life Science and Technology, Huazhong University of Science and Technology

Disturbed inhibitory synaptic transmission has functional impacts on the pathology of neurodevelopmental and psychiatric disorders (including autism spectrum disorders and depression). An essential mechanism for modulating inhibitory synaptic transmission is the alteration of the postsynaptic abundance of $GABA_A$Rs. The synaptic $GABA_A$Rs are stabilized by postsynaptic scaffold proteins such as NLG-1/Neuroligin and FRM-3, and also can be recruited by the presynaptic signals during synaptogenesis and activity-dependent synaptic plasticity. However, how presynaptic GABAergic neurons trigger signals to transsynaptically recruit $GABA_A$Rs remains elusive. Here, we show that UNC-43/CaMKII functions at GABAergic motor neurons to recruit $GABA_A$Rs and modulates inhibitory synaptic transmission at *C. elegans* neuromuscular junctions (NMJs). We demonstrate that UNC-43 promotes presynaptic MADD-4B/Punctin secretion and facilitates NRX-1a/Neurexin GABAergic motor neuron surface delivery. Together, MADD-4B and NRX-1α recruit postsynaptic scaffold protein NLG-1/Neuroligin and stabilize $GABA_A$Rs. Further, during the induction of activity-dependent plasticity at inhibitory synapses, we found that excitation of GABAergic neurons potentiates the recruitment of NLG-1-stabilized $GABA_A$Rs—but not FRM-3-stabilized $GABA_A$Rs—showed that such recruitment depends on the UNC-43, MADD-4B, and NRX-1. These lines of evidence all support that UNC-43 triggers MADD-4B and NRX-1α, which act as anterograde signals to recruit postsynaptic NLG-1 and to stabilize $GABA_A$Rs at inhibitory synapses. Thus, our findings elucidate a mechanism for pre- and postsynaptic communication and inhibitory synaptic transmission and plasticity.

828B **Sexual dimorphism in PVD neuron dendritic branching and its effect on male mating** Yael Iosilevskii, Benjamin Podbilewicz Technion - Israel Institute of Technology

The PVD sensory neuron in *C. elegans* is polymodal, involved in the perception of noxious touch, temperature, sound vibrations and body position. Its highly arborized structure forms multiple candelabra-shaped units post-embryonically, which are maintained in early adulthood. PVD structure is stereotypical, with distinct symmetrical and asymmetrical features, offering a convenient model for studying dendritic patterning, maintenance, and repair mechanisms. While multiple genes are known to affect hermaphrodite PVD morphology, male PVD structure remains less characterized. In particular, the *C. elegans* male tail is an intricate structure containing 9 ray pairs supporting a cuticular fan, presenting a compelling model for functional organ morphogenesis and innervation. Each ray of the male tail contains two neurons, RnA and RnB (n = 1-9), which assist in guiding correct mating behavior. We show that, in addition to these two neurons, PVD also increasingly extends dendritic branches into the tail rays in early adulthood. In order to characterize the effects of PVD tail innervation and presence in male mating, we utilized mutant backgrounds *mec-3 (e1338)* and *sax-7(dz156)*, known to influence hermaphrodite PVD patterning, as well as complete genetic ablation of the PVD. PVD morphology in male *mec-3(e1338)* and *sax-7(dz156)* mutants resembles known hermaphrodite morphology. These mutants display some mating defects in turning, possibly compounded by additional effects on multiple neurons. Complete and specific genetic ablation of PVD causes defects in several steps during male mating behavior. Our results reveal a possible role for PVD in mating proprioception, and suggest a new avenue for studying organ innervation during early adulthood.

829B **Multiple Neuronal Signals Regulate the Plasticity of A Key Modulatory Neuron in A Memory Circuit** Wai Hou Tam, Chun-Liang Pan Institute of Molecular Medicine and Center for Precision Medicine, National Taiwan University

Physiological stress can induce the formation of aversive memory for contextual sensory cues. We recently showed that monoaminergic neuromodulation regulates *C. elegans* aversive memory under systemic mitochondrial stress. The RIC octopaminergic neuron responds to systemic stress by upregulating octopamine synthesis and is critical for the formation of aversive memory. Bacterial cues evoke RIC responses in stressed but not naive animals. As RIC does not form synapses with most sensory neurons, we hypothesize that stress activates functional communications between the sensory neurons and RIC. To identify neuronal signals that remodel RIC responses under mitochondrial stress, we screened mutants that lack neurotransmitters, biogenic amines and neuropeptides. Sensory-evoked RIC activities require octopamine and GABA, as well as the SER-6 octopaminergic receptor, which is expressed in RIC and several neurons of the memory circuit. Thus, RIC responses are likely regulated by autocrine octopamine signaling, which may serve to sensitize RIC to stress. By contrast, acetylcholine, glutamate, and neuropeptides have more complex roles in RIC dynamics: they promote evoked RIC activities under stress but prevent RIC activation by sensory cues in stress-free animals. As RIC has few synapses with cholinergic and glutamatergic neurons, we hypothesize that extrasynaptic acetylcholine and glutamate signaling is a major mechanism for RIC modulation. Consistent with this idea, we found that the MGL-2 metabotropic glutamate receptor is required for evoked RIC activities and stress-induced aversive memory. Our findings uncover signaling mechanisms that reconfigure functional connections to a key modulatory neuron in the formation of stress-induced memory. (Supported by National Science and Technology Council MOST 109-2320-B-002 -019 -MY3)

830B **Restless legs syndrome drug screen in *C. elegans* model.** Rachel De Barros Oliveira[1], Patrick Dion[2,3], Alex Parker[4,5], Guy Rouleau[2,3,61]McGill University, [2]Department of Neurology and Neurosurgery, McGill University, [3]Montreal Neurological Institute and Hospital, [4]Département de Neurosciences, Université de Montréal, [5]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), [6]Department of Human Genetics, McGill University

Restless legs syndrome (RLS) is a chronic sleep-related sensorimotor disorder characterized by a strong impulse to move the legs and relieve uncomfortable sensations. In 2007, a genome-wide association study identified significant associations between RLS and three genomic regions, one of which comprises a highly significant intronic variant in the homeobox gene *MEIS1*. Carriers of this particular *MEIS1* variant have a 50% increased risk of developing RLS. Using a simple and strong genetic model organism *Caenorhabditis elegans* our team previously reported reduced expression of *UNC-62* (*C. elegans* ortholog of *MEIS1*) to be associated with iron homeostasis via an increased expression of ferritin ortholog (FTN-1). The *unc-62* orthologue is expressed in different tissues (hypodermis, intestine, and nervous system) and its downregulation led to a strong movement phenotype. Different *unc-62* alleles showed defects in *cat-2/tyrosine hydroxylase* (involved in dopamine synthesis). In our work, we took advantage of the mobility difference between the *unc-62(e644)* strain and the control strain (*N2*) and performed an unbiased drug screen. We identified 21 compounds (from a library of ~4,000 compounds) showing promise in their ability to rescue the mobility of *unc-62(e644)*. The benefits and impact of these compounds are under validation. To correlate the dopaminergic system and iron expression, we generated *unc-62* strains expressing GFP::DAT-1 and GFP::FTN-1 to monitor their expression. Our work highlights the advantages of using the unc-62 model to evaluate other genes and pathways involved in RLS.

831B **Rapid detection and recognition of whole brain activity in a freely behaving *Caenorhabditis elegans*** Yuxiang WU, Tianqi Xu, Quan WenNeurobiology, University of Science and Technology of China

Advanced volumetric imaging methods and genetically encoded activity indicators have permitted a comprehensive characterization of whole brain activity at single neuron resolution in *Caenorhabditis elegans*. The constant motion and deformation of the nematode nervous system, however, impose a great challenge for consistent identification of densely packed neurons in a behaving animal. Here, we propose a cascade solution for long-term and rapid recognition of head ganglion neurons in a freely moving *C. elegans*. First, potential neuronal regions from a stack of fluorescence images are detected by a deep learning algorithm. Second, 2-dimensional neuronal regions are fused into 3-dimensional neuron entities. Third, by exploiting the neuronal density distribution surrounding a neuron and relative positional information between neurons, a multi-class artificial neural network transforms engineered neuronal feature vectors into digital neuronal identities. With a small number of training samples, our bottom-up approach is able to process each volume—1024 × 1024 × 18 in voxels—in less than 1 second and achieves an accuracy of 91% in neuronal detection and above 80% in neuronal tracking over a long video recording. Our work represents a step towards rapid and fully automated algorithms for decoding whole brain activity underlying naturalistic behaviors.

832B **RIM neurons integrate sensory and motor signals to modulate chemotaxis strategy** Talya S Kramer, Flossie K Wan, Sarah M Pugliese, Jinyue Luo, Steve Flavell MIT

When animals navigate sensory gradients, they must weigh sensory information from their past and present surroundings to determine when to change direction. Previous work on *C. elegans* chemotaxis has identified motor strategies used to approach or avoid odors, as well as sensory and interneurons underlying these calculations. However, whether these strategies are constant over time or can change depending on context is unknown. Here, we show that tyraminergic modulation dynamically adjusts animals' chemotaxis strategy as they navigate odor gradients. A candidate genetic screen revealed that animals lacking the neurotransmitter tyramine, which is released from the neuron pair RIM, have impaired behavioral responses to attractive and aversive odors. High-resolution recordings during olfactory navigation identified two novel, tyramine-dependent chemotaxis strategies: wild-type animals initiate pirouettes after failing to correct their bearing in the odor gradient with single reversals and preferentially terminate pirouettes when facing in a favorable direction in the odor gradient. To examine how RIM tyramine modulates pirouettes based on sensory information, we performed RIM calcium imaging in freely-moving animals navigating odor gradients. These recordings show that RIM activity is highest during long, fast, consecutive reversals, such that the largest RIM peaks occur during pirouettes. In addition, RIM activity is modulated by the animal's location and movement in the odor gradient; these sensory representations in RIM require input from the interneuron AIB. Finally, we find that tyramine acts to amplify RIM's activity, providing a positive feedback loop that may allow tyramine to elongate reversals or promote pirouettes. We propose a working model wherein RIM integrates the animal's recent movement and olfactory context, displaying highest activity after unfavorable movement in a gradient. RIM then signals via dynamic tyramine release to promote reversals until the animal is in a favorable direction in the odor gradient. This dynamic change in chemotaxis strategy, integrating recent movement and olfactory context, may allow animals to navigate in complex olfactory gradients.

833B **Peripheral Peroxisomal Lipid Signaling Targets a Serotonergic Neuron to Regulate Stress-Induced Aversive Memory in *C. elegans*** Shang-Heng Tsai, Yu-Chun Wu, Chun-liang PanInstitute of Molecular Medicine and Center for Precision Medicine,

Disruption of physiological homeostasis elicits cellular stress response and behavioral adaptation of the animal. We recently showed that mitochondrial stress induces aversive associative memory for food bacteria that *C. elegans* inherently prefers without stress. We now provide evidence that mitochondrial stress triggers the formation of aversive memory through peroxisomal lipid metabolism in peripheral tissues. Genes for peroxisomal β-oxidation, including *daf-22*, *dhs-28* and *pmp-4*, which encodes a transporter of very long chain fatty acid (VLCFA), are required for stress-induced aversive memory, and they are upregulated by mitochondrial stress. Peroxisomal β-oxidation genes function in the intestine and hypodermis but not neurons, and *pmp-4* is required for aversive memory formation. Peroxisomal metabolic signals target the NSM neuron to increase serotonin synthesis and induce sensory-evoked calcium dynamics under stress. As NSM and serotonin are important for the establishment of stress-induced aversive memory, our current study suggests that gut-brain peroxisomal signaling connects peripheral mitochondrial stress and a neuromodulatory circuit to drive stress-induced memory and avoidance behavior. (Supported by the National Science and Technology Council, MOST 110-2320-B-002 -057 -MY3)

834B **Transcriptomic Analysis and Candidate Screens for Genes in Stress-Induced Aversive Memory** YEN-JU CHEN[1], Shyang-Jen Wu[1], Yu-Chun Wu[1], Yu-Cheng Tang[1], Yueh-Chen Chiang[21]Institute of Molecular Medicine and Center for Precision Medicine, National Taiwan University, [2]Johns Hopkins University

Aversive associative memory is a critical strategy for animals to avoid environmental threats. Physiological stress, including mitochondrial disruption, induces aversive memory. We recently established a *C. elegans* model of aversive associative memory triggered by mitochondrial disruption. Here we profile gene expression patterns for the formation and retrieval of this stress-induced aversive memory. We find that neuronal genes important for synaptic signaling and calcium homeostasis are progressively activated during memory formation and reactivated in memory retrieval. We detected genes specifically upregulated in either the formation or retrieval of memory, implying distinct genetic basis for either process. To confirm the importance of upregulated neuronal genes in memory functions, we conducted a candidate mutant screen focusing on those that are also CREB targets. Among several positive hits, we identified *casy-1*/ calsyntenin to be important for stress-induced aversive memory. CASY-1A, which retains the extracellular domains, and CASY-1B that lacks the extracellular domains are expressed in different subsets of neurons. When expressed from their respective promoters, both *casy-1a* and *casy-1b* rescued memory defects of the *casy-1* mutant. Rescue experiments that swapped the promoters of *casy-1* isoforms showed that CASY-1A can compensate for the function of CASY-1B, but CASY-1B failed to rescue when expressed from the *casy-1a* promoter. Our work serves as a foundation to further probe the genetic mechanisms of stress-induced aversive memory.

835B **Role of Neuromodulation in CASY-1 mediated regulation of locomotion in *C. elegans.*** Navneet Shahi, Kavita Babu Centre for Neuroscience, Indian Institute of Science

Locomotion is a basic yet an indispensable process for the survival of a variety of organisms. It is thus highly regulated by a network of neuronal circuits, responsible for maintaining the excitation-inhibition (E-I) balance at neuromuscular junction (NMJ). Several cell adhesion molecules (CAMs) have been found to act as dynamic regulators of locomotory circuit function. CASY-1, is one such CAM present in the neurons of nematode *Caenorhabditis elegans* (*C. elegans)*, known to coordinate locomotion by maintaining E-I balance at NMJ. It has been demonstrated that the *casy-1* mutants display accelerated motor activity and paralysis in response to Aldicarb (acetylcholinesterase inhibitor), suggesting higher cholinergic signaling. However, the exact mechanism by which the CASY-1 present at the sensory and interneuron level acts to regulate cholinergic signaling in the motor neurons is poorly understood. Numerous studies report the role of neuropeptides in modulating E-I balance in the locomotory circuit. Also, neuropeptide processing *egl-3* and *egl-21* mutants are found to suppress the Aldicarb hypersensitivity of *casy-1* mutants. Moreover, Preliminary experiments have indicated the genetic interactions between *casy-1* and the neuropeptide *flp-21* and its receptor *npr-1*, based on Aldicarb and locomotion assays. This led us to hypothesize that CASY-1 possibly acts through neuropeptidergic signaling to regulate sensory-evoked motor output. In order to understand the intricate neuromodulatory mechanisms operating in the locomotory circuit, *C. elegans* is employed as a model system since its neural connectome has been studied in detail. Hence, we aim to establish the neuropeptidergic circuit, responsible for CASY-1 mediated regulation of motor circuit dynamics and associated locomotory behavior.

836B **The cell surface receptor GOGO-1 controls follower axon navigation in the ventral nerve cord** Debapriya Roy, Jie Pan, Harald Hutter Biological Sciences, Simon Fraser University

Development of neuronal connection starts with axon outgrowth. The first outgrowing axons (“pioneers”) pave the initial pathways, which are then used by later outgrowing axons (“followers”) to reach their target cells. Previously, we have established the role of a non-classical cadherin, *fmi-1,* in pioneer-follower axon navigation [1]. *fmi-1* mutants show a characteristic phenotype in the ventral nerve cord, where the PVQL follower axon uncouples from the pioneer axon PVPR resulting in axon navigation errors.

Interestingly, the intracellular domain of *fmi-1* is not necessary for PVQ axon navigation. Therefore, we hypothesized that *fmi-1* acts with a co-receptor. In *Drosophila*, Golden Goal is a co-receptor for the FMI-1 homolog Flamingo in axonal targeting in the visual system. We found that mutations in *gogo-1* disrupt the pioneer-mediated navigation of the PVQL follower axon, which no longer follows the PVPR pioneer axon and aberrantly crosses the ventral midline. Both PVP and PVQ axons show ventral midline crossover defects, but PVQ axon crossovers are almost twice as frequent as PVP axon crossovers. This suggests that *gogo-1* is independently also required for PVQ follower axon navigation. These phenotypes are qualitatively similar to defects in *fmi-1* mutants, but less penetrant in *gogo-1* mutants. Mutations in both genes also cause HSN axon navigation defects such as looping around the vulva, and axons projecting to the posterior instead of the anterior. All affected neurons express *fmi-1* and we have shown that FMI-1 acts cell-autonomously in PVP and PVQ [1]. To determine whether *gogo-1* is also expressed in PVP and PVQ, we tagged GOGO-1 at the C-terminus with GFP using CRISPR/Cas9. The resulting strain only showed weak expression in the nerve ring, which probably does not reflect the complete expression pattern of *gogo-1*. To determine the cellular expression of *gogo-1,* we currently employ an operon-tagging strategy expressing soluble GFP together with native *gogo-1*. So far, we identified an important role for *gogo-1* in both pioneer and pioneer-dependent follower axon navigation. Further experiments are in the pipeline to confirm that *gogo-1* is a co-receptor of *fmi-1*.

1. Steimel et al., Development 137(21):3663-73 (2010)

837B **Neurogenetic mechanisms underlying sexually dimorphic behavioral states in *C. elegans*** Gregory Reilly[1], Chance Bainbridge[2], Jinxin Wang[1], Douglas S Portman[1,21]Neuroscience, University of Rochester, [2]Biomedical Genetics, University of Rochester

Biological sex is a fundamental dimension of internal state that can have deep influences on behavior. Understanding the mechanisms behind these influences can provide insight into how shared neural circuits are tuned to produce sex-specific behavioral variation. Biological sex can influence both short-term behaviors and longer, more persistent forms of behavior known as behavioral states. In *C. elegans*, persistent motor behavior, called locomotor states, is well-studied in hermaphrodites. On a patch of food, hermaphrodites will stochastically switch between two states: roaming and dwelling. However, while some work has examined motor states in males, these remain poorly characterized. Our lab has shown that locomotor behavior is sexually dimorphic; the sexual state of both nervous and muscle tissue is essential for sex-characteristic body posture and speed. Therefore, biological sex may also similarly influence locomotor states. We trained a supervised machine learning Random Forest model to detect three locomotor states: roaming, dwelling, and tail chase. While both males and hermaphrodites share the locomotor states of roaming and dwelling, the characteristics of these differ by sex- the amount of time spent in each state and the transition probabilities between states display sexual dimorphisms. To understand how sex tunes these locomotor states, we manipulated the sex determination pathway to sex reverse the nervous system in both males and hermaphrodites. Interestingly, we found that the locomotor states of pan-neuronally feminized males had characteristics of wildtype hermaphrodites- the time spent in each state, and transition probabilities between states were like wildtype hermaphrodites. Preliminary analysis also suggests masculinized hermaphrodites showed similar locomotor state characteristics to wildtype males. Furthermore, initial data using animals in which subsets of neurons were sex-reversed indicates the sexual identity of the sensory neurons is required for sex-characteristic locomotor states. To uncover the mechanisms that biological sex leverages to achieve this sex-specific variance in locomotor states, we are testing mutants for neuromodulatory pathways. Preliminary data suggest that sex differences in the PDFR-1 signaling pathway contribute to sexual dimorphism in locomotor states. Together, our results provide a mechanistic framework for understanding how sex-specific neuronal tuning influences behavioral states.

838B **Decoding the role of novel memory regulators CEY/YBX RNA binding proteins in neurons** Ashley Hayden[1], Katie Brandel-Ankrapp[1], Edward Pietryk[2], Rachel Arey[31]Neuroscience, Baylor College of Medicine, [2]Genetics, Baylor College of Medicine, [3]Molecular and Cellular Biology, Baylor College of Medicine

RNA binding proteins regulate key aspects of RNA metabolism, including the translation of target mRNAs. Translational control of mRNAs is especially important in the nervous system, as novel protein synthesis is necessary for many types of behavior. However, despite their critical biological significance, many RNA binding proteins within the nervous system remain uncharacterized for roles in neuronal function, especially in the context of behavior. Using transcriptomic datasets from the adult nervous systems of both mammals and *C. elegans*, we identified the CEY/YBX RNA binding proteins (CEY-1, CEY-2, CEY-3, and CEY-4) as a conserved protein family that has neuronal expression across species but has no known role in the adult nervous system. To determine whether CEY RNA binding proteins have an important role in cognitive health, we first tested whether adult-only, neuron-specific knockdown of each CEY RNA binding protein decreases positive olfactory associative memory in *C. elegans*. We identified three of the four CEY RNA binding proteins as novel regulators of associative memory. Specifically, we found that each CEY family member regulates its own mechanistically distinct form of memory (short-term, intermediate-term, or long-term memory). Because the CEY RNA binding proteins are orthologs of Y-Box Binding Proteins (YBX's) in mammals, we next examined whether dysfunction of YBX1, YBX2, and/or YBX3 are associated with neuronal symptoms in humans. Using a combination of publicly available as

well as institute-specific human variant datasets, we found that a large proportion of patients with variants in any of the three YBX RNA binding proteins have severe neurological symptoms. Importantly, the most common neurological symptom reported in these patients is intellectual disability, mirroring our findings in *C. elegans*. We have uncovered a previously unappreciated role for the CEY/YBX RNA-binding proteins in neuronal and cognitive health. In ongoing work, we are investigating the role of these proteins in adult *C. elegans* neurons using a combinatorial approach of phenotypic batteries, transcriptomics, microscopy, and transgenic modeling. Our goal is to identify mechanisms in *C. elegans* that can inform the molecular underpinnings of YBX-related neurological symptoms in human patients and learn more about how neuronal RNA binding proteins control cognitive health and behavior.

839B **Sensory encoding of temporal gradients** Maggie M Chang, Michael Hendricks Biology, McGill University

In animal behaviour, sensory encoding is a critical process for survival that allows the animal to sense changes in its environment and respond accordingly through behavioural outputs. Locomotion driven by sensation is a crucial aspect of navigation that is important in essential processes such as foraging and avoiding noxious stimuli. Calcium responses in sensory neurons have primarily been studied in response to step stimulus changes or long, steady changes in stimulus concentration, neither of which capture the spatiotemporal fluctuations of natural stimuli. For example, animals sense and respond both to concentration changes over tens of seconds as they move through gradients, but also to the smaller, faster fluctuations driven by head movements. To create this in a laboratory setting, we are designing a microfluidic flow control system device that can deliver smoothly graded fast and slow temporal stimulus fluctuations that can easily ramp up or down in concentration, and importantly, generate gradients that mimic sensory input encountered during navigation behavior on plates. With this system, we will observe how sensory neurons respond to various graded stimuli using calcium imaging. This platform allows us to deliver either reproducible or random noise stimulus fluctuations to restrained animals to test a broad range of stimulus parameters on sensory neuron function, unlike freely moving animals, where stimulus changes depend on the animal's behaviour. This project will further our understanding of sensory encoding, the critical first step in animal navigation.

840B **Phenomic Characterization of *C. elegans* orthologs of Parkinson's Disease-Associated Genes** Joseph Liang[1], Jessica Chalissery[1], Kristen Tsoi[1], Ben Westmore[1], Catharine Rankin[21]University of British Columbia, [2]Psychology, University of British Columbia

Parkinson's Disease (PD) is a neurodegenerative disease that effects more than 1 million people worldwide. Our current understanding of the genetic contributions of PD has been expanded by advances in genome wide association studies (GWAS) in the past decade, but efforts in the functional characterization of newly identified risk loci have lagged behind the rate of new discoveries. To address this issue, I established a pipeline for *in vivo* characterization of orthologs of newly identified PD risk loci. *C. elegans* is ideal because: 1) *C. elegans* have orthologs to many PD-associated and biologically relevant genes. 2) *C. elegans* researchers have curated a library of strains with loss-of-function mutations available for almost every gene in the *C. elegans* genome including many identified in the PD-GWAS studies. 3) Our lab developed the **Multi-Worm Tracker (MWT)** for high-throughput characterization of behavioural and morphological phenotypes in populations of freely behaving animals in real time. Phenotyping strains with mutations in orthologs of PD-linked will yield unique phenotypic profiles for each disease-linked gene, and further analyses of these profiles may yield further insights on novel gene interactions or molecular pathways enriched involved in PD. A list of 180 mutant strains harbouring mutations in 83 *C. elegans* genes that are orthologous to 53 PD-linked genes, a majority of which are previously uncharacterized for biological function and disease relevance will be characterized across an array of 30 phenotypes ranging from the Basal Slowing Response, a DA-dependent behaviour, and habituation, the simplest form of learning (altered in PD patients), to baseline morphology and behaviour including but not limited to locomotion speed and animal width and length. Based on the hypothesis that genes functioning in the same molecular pathway will lead to similar or strongly opposite phenotypic profiles when mutated compared to those that are not, I will also perform hierarchical clustering of phenomic profiles to predict novel gene interactions and uncover potential molecular pathways involved in PD. This research will characterize PD-linked genes in a dopamine-dependent behaviour, establish high-throughput genotype-to-phenotype characterization of newly identified risk genes for PD, and identify new functional interactions and gene networks to inform future disease modelling efforts and further our understanding of the biological processes underlying PD.

841B **Investigating the role of a neural bottleneck in *C. elegans*** Elsa Bonnard, Jun Liu, Monika Scholz Neural information flow, Max Planck Institute for Neurobiology of Behavior

A neural bottleneck is characterized by the projection of multiple neurons onto a smaller number of neurons. This architecture suggests that the network compresses information encoded in the incoming signals. However, no direct measurement of all neurons involved in a bottleneck motif has been realized so far. Such a measurement would allow us to experimentally determine how a neuronal network performs information compression. The pair of RIP neurons in *C. elegans* represents a simple implementation of a neural bottleneck where such measurements are possible. RIP neurons receive converging sensory inputs and

provide the only connections to the pharyngeal network controlling feeding through gap-junctions with the pair of pharyngeal I1 neurons. Harsh touch inhibits pumping, a characteristic pharyngeal muscle motion involved in feeding and mechanoreceptor neurons are upstream of the bottleneck. To investigate the role of the RIP-I1 bottleneck, we supply touch stimuli as input signal while observing pumping as behavioral read-out. Estimating information compression requires a large amount of measurements of both the input and output signals. Therefore, we implemented a high-throughput assay to supply substrate vibrations as touch stimulus while observing pumping in *C. elegans* populations. Using our custom image analysis pipeline 'PharaGlow', we can automatically detect pumping events in tens of animals moving on standard cultivation plates. We found that pumping is inhibited by vibrations in an intensity dependent manner. This inhibition is abolished in the touch defective mutants *mec4(u253).* The *mec4* gene codes for a subunit of the mechanotransducer channel present only in the 6 touch receptor neurons (TRNs). These results give us access to the input layer of the bottleneck. We will discuss how we are testing the requirement of the RIP-I1 bottleneck to transfer the touch signal to the pharynx by genetically ablating I1 neurons using the human caspase interleukin-1β-converting enzyme (ICE) and by silencing the I1 neurons using targeted expression of histamine-chloride channels. We further compare the pumping inhibition in the presence and absence of attractive food cues to test if the integration of sensory information at the bottleneck is context-dependent.

842B **Whole brain imaging of freely moving C. elegans under a thermal stimulus** Core Francisco Park, Helena Casademunt, Vladislav Susoy, Aravinthan SamuelPhysics, Harvard University

With recent advances in confocal imaging and neuron labeling, we can now record from a majority of the worm's brain during freely moving behavior. Additionally, we have developed automated neuron tracking for high throughput analysis. Here, we present an analysis of 40+ neurons in 20+ animals during freely moving behavior under a thermal stimulus. We condition worms at different temperatures and expose them to sinusoidal temperature waves. First, we confirm previously known responses of the AFD and AVA neuron to thermal stimulus. Next, we analyze neural activity to predict some aspects of the behavior and vice-versa. Third, we present findings from the same experiment with mutant strain lacking all gcy-23, gcy-8, gcy-18 which thus has severe defects in thermotaxis. Finally, we discuss preliminary findings about the BAG neuron and its relation to behavior under a thermal stimulus.

843B **Regulation of Synaptic Development at Neuromuscular Junction via PXF-1 and RAC-2 Signaling** Reagan Lamb, Salvatore J Cherra Neuroscience, University of Kentucky

The family of Rho GTPases is comprised of important modulators of cellular and molecular signaling. Of these Rho GTPases, the Rac *Caenorhabditis elegans* orthologues, RAC-2, CED-10, and MIG-2, are known to have a wide range of functions within neurons including axonal guidance, mechanisms governing forgetting, and cell migration. While they are found to be triply redundant in some pathways, new studies have shown that they also may act independently of one another in cell and tissue specific manners. We previously found that the *C. elegans* PDZ-GEF orthologue, PXF-1, promotes the development of synapses at the neuromuscular junction. We found that *pxf-1(gk955083)* mutants animals exhibited decreased synaptic vesicle intensity, frequency of calcium transients, and perisynaptic filamentous actin. Based on the canonical role of Rho GTPases in regulating cytoskeletal development, we hypothesized that PXF-1 may be acting within a GTPase signaling pathway upstream of a known RAC protein. To determine this, we again used the neuromuscular junction of *C. elegans* as our model circuitry system. We first screened multiple GTPases mutants for their sensitivity towards the acetylcholinesterase inhibitor, aldicarb, and found that *rac-2(ok326)* and *ced-10(n1993)* mutant animals displayed resistance to aldicarb as compared to wild type animals. Focusing on RAC-2, we next examined the synaptic vesicle intensity of *rac-2(ok326)* and *pxf-1(gk955083)* single and double mutant animals using mCherry::RAB-3. We found that each single mutant and the double mutants all displayed decreased mCherry::RAB-3 intensity. We also found that mutations in the canonical RacGEF, TIAM-1, using *tiam-1(1556)* caused a similar decrease in mCherry::RAB-3 intensity in the presence and absence of the *pxf-1(gk955083)* mutation. Using a GFP::utrophin-calponin homology domain (GFP::ut-CH) as a marker for filamentous actin, we measured perisynaptic GFP::ut-CH intensity in *rac-2(ok326)* and *pxf-1(gk955083)* single and double mutants and found again a significant decrease in GFP::ut-CH intensity in all three mutants compared to wild type. Finally, to better determine whether *pxf-1* mediated defects are caused by a decrease in RAC-2 activity, we pan-neuronally expressed wild type RAC-2 and constitutively active RAC-2 G12V in the *pxf-1(gk955083)* mutant background. We found that expression of constitutively active, but not wild type, RAC-2 was able to restore synaptic vesicle intensity to wild type levels. Overall, these findings indicate that RAC-2 may act downstream of PXF-1 to mediate its effects on synapse development. These findings will contribute to a greater understanding of neuronal function and developmental signaling.

844B **Integrate or Disintegrate: understanding the neuronal mechanisms underlying sensory integration in *C. elegans*** Caroline S Muirhead, Jagan Srinivasan Biology and Biotechnology, Worcester Polytechnic Institute

All animals, including humans, regularly encounter competing sensory information. The brain coordinates these conflicting signals so that animals can respond accordingly to the environment. This process, known as sensory integration, is important because it underlies higher order cognitive function. The sensory integration process is complex and therefore, difficult to study.

Using *C. elegans,* we are able to study sensory integration at a single cell level by exposing them to conflicting sensory cues and interrogating how their neurons integrate the differential cues. Worms are attracted to *E. coli* because it is their food source. An opposing cue to *E. coli* extract is osas#9. osas#9 is a pheromone released by starved L1 worms. Because osas#9 communicates that food is not present, starved worms avoid this cue. However, we found that mixing *E. coli* extract into osas#9 attenuates starved worms' innate avoidance of osas#9. The behavioral shift from avoidance to non-avoidance with the addition of food allows us to study how these differential cues are integrated by the nervous system. The change in behavior created by the presence of *E. coli* extract suggests that the *E. coli* cue is canceling the osas#9 avoidance signal. osas#9 avoidance attenuation is a concentration-dependent process, suggesting that cue strength is also weighed by the worm. We will present data showing that serotonin signaling and the ADF neurons are required for the nervous systems integration of osas#9 and food cues.

845B **Temperature-mediated chemotactic behavioral plasticity by aversive olfactory conditioning in C. elegans** Nour Halaby Biology, McGill University

C. elegans are capable of different types of learning (associative, non-associative and imprinting), despite the fact that they have a nervous system comprised of only 302 neurons. This is possible because of their not so simple neural circuitry, which consists of 2000 neuromuscular junctions, 700 gap junctions and around 5000 synapses. Accordingly, the changes in the components of the neural circuit in terms of neural excitability and synaptic strength alter information flow to the circuit and support diverse behavioral outputs for the same sensory input. The associative learning mechanisms linking the molecular level and the circuit activity level are not fully understood. Temperature has been well-characterized as a conditioned stimulus paired with food in appetitive associative learning. Here, we use noxious temperature instead as the unconditioned stimulus to study aversive olfactory learning and imprinting. Neurons implicated in this conditioning will be identified through genetically-targeted inactivation and calcium imaging, and gene expression profiling will be used to identify candidate molecular mechanisms.

846B **Cross-tissue tuning of neuronal proteostasis by an intestinal molecular chaperone and microRNA in *C. elegans*** Yang Li[1,2], Yingchun Ni[1,2], Yinghui He[1,2], Yuzi Wang[3], Weiqi Zhao[1,2], Xing Guo[4], Zhiping Wang[41]Department of Neurobiology and Department of Neurology of Second Affiliated Hospital, NHC and CAMS Key Laboratory of Medical Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, China, [2]The MOE Frontier Science Center for Brain Research and Brain-Machine Integration, Zhejiang University School of Brain Science and Brain Medicine, Hangzhou 310058, China, [3]College of Life Sciences, Zhejiang University, Hangzhou 310058, China, [4]Zhejiang Provincial Key Laboratory for Cancer Molecular Cell Biology, Life Sciences Institute, Zhejiang University, Hangzhou 310058, China

In multicellular organisms, inter-tissue regulation of proteostasis has been gradually recognized as an important strategy to coordinate environmental and intrinsic cues for better viability and sustainability. To systematically examine how protein quality control (PQC) components are organized at the organismal level to guard neurodevelopment, we performed an RNAi screen for PQC genes required for intertissue regulation of axon guidance in *C. elegans*. Interestingly, a set of non-neuronal PQC genes showed differential regulation of AVM and HSN, which mature at different larval stages. Further, we demonstrated that intestinal cells were capable to deliver materials to HSN neurons. This process depended on a previously rarely studied intestinal ER chaperone DNJ-2 and a stress-responding intestinal microRNA *mir-60*. Deletion of *dnj-2* or *mir-60* impaired HSN guidance accuracy, while intestinal or neuronal overexpression of *mir-60* rescued these defects. Mechanistic studies revealed that DNJ-2 and its mammalian homolog DNAJC25 regulate biogenesis and secretion of exosomes from late endosomes (LEs). During HSN development, exosomes containing *mir-60* secreted from the intestine facilitated folding of essential guidance receptors, including SAX-3 and UNC-40, and suppressed potential guidance errors under suboptimal growth conditions. Together, our findings have elucidated general principles of cross-tissue proteostatic regulation and uncovered a new PQC mechanism involving chaperone-dependent release of exosomes and miRNAs.

847B **Analysis of the effect of *unc-11* on the aggregation and pathology in *C. elegans* Alzheimer disease models** Mira Sleiman[1], Gurleen Kaur Kalsi[1], Sophie Kothe[1], Franziska Hirsch[1], Sara Maria Ayala Mariscal[2], Domenico Arzania Tehran[2], Tanja Maritzen[3], Janine Kirstein[1,41]University of Bremen, [2]Leibniz Institute for Molecular Pharmacology, [3]Technical University of Kaiserslautern, [4]Leibniz Institute on Aging – Fritz Lipmann Institute

Alzheimer's disease (AD) is a progressive neurodegenerative disease manifested as cognitive and behavioral impairments. There are two main molecular biomarkers of AD, the extracellular amyloid beta (Aβ) plaques and the intracellular hyperphosphorylated tau tangles. Age and genetic mutations are the major risk factors of AD. A recent genome-wide association studies (GWAS) have identified the gene coding for the Phosphatidylinositol-binding clathrin assembly protein (PICALM/CALM) as a risk factor of the late onset AD. CALM is known to be involved in the clathrin-dependent endocytosis regulating the size and density of synaptic vesicles. Nevertheless, its role in AD pathology is not understood.

We can demonstrate that CALM and AP180, a neuron-specific CALM homolog, suppress $A\beta_{1-42}$ aggregation. Employing a Thio-

flavin T assay to study the kinetic of amyloid fibrils, an increase of fluorescence was detected after approximately 30 hours, indicating an increase of aggregation. Upon addition of either CALM or AP180 to $A\beta_{1-42}$, the fluorescence was suppressed, suggesting a strong and direct inhibitory effect of CALM and AP180 on $A\beta_{1-42}$ aggregation. To test this hypothesis *in vivo*, we took advantage of our amyloid beta model to create a new strain simultaneously carrying an *unc-11*, a CALM homolog in *C. elegans*, loss of function mutation and expressing, in a pan neuronal way, the human $A\beta_{1-42}$ peptide tagged with wrmScarlet or, as control, wrmScarlet alone.

Compared to the neuronal Aβ strain carrying the wild type *unc-11* allele, the new strain showed an equally reduced lifespan, a drastic reduction in brood size and an impaired organismal fitness. Interestingly, fluorescence lifetime imaging microscopy (FLIM) analyses showed a significantly higher age-dependent $A\beta_{1-42}$ aggregation upon *unc-11* mutation, confirming our *in vitro* data. In conclusion, we could show that UNC-11 plays an important role in AD pathology regulating Aβ aggregation.

Finally, to explore the full potential of UNC-11 as a putative therapeutic target in AD, we recently generated an *unc-11* overexpressing strain and are currently investigating its effect on Aβ proteotoxicity and will expand our analysis to tau, using our novel unpublished Tau model.

848B **Syndecan, netrin, guidance receptors and Rho-family GTPases cooperate to regulate the number of cellular extensions** Raphael DIMA[1], Marianne Bah Tahé[1], Yann A Chabi[1], Lise Rivollet[1], Anthony F Arena[2], Alexandra M Socovich[2], Daniel Shaye[2], Claire Bénard[1,3]1Biological Sciences, CERMO-FC Research center, Université du Québec à Montréal, [2]Physiology and Biophysics, University of Illinois at Chicago, [3]Neurobiology, University of Massachusetts Medical School

During development, neurons and other polarized cells establish precise morphologies that are critical to their function. Whereas much progress has been made towards understanding the mechanisms driving axon outgrowth and guidance, how neurite *number* is regulated in a developing neuron remains unknown. Our analysis of mutants in heparan sulfate proteoglycans (HSPGs) has revealed the existence of a mechanism controlling neurite/cellular extension number. HSPGs are glycoproteins that regulate interactions between extracellular signals and their receptors to orchestrate cellular responses. HSPGs consist of a protein core with attached heparan sulfate (HS) glycosaminoglycan chains. Mutants in the two C. elegans HS glycosyltransfereases, *rib-1* and *rib-2*, display a striking defect that has been rarely observed: neurons that are unipolar in the wildtype can develop two neurites from the soma, and the excretory canal cell can develop up to 8 canals instead of 4. Since axons and the excretory canals rely on common molecular mechanisms for the guidance of their migration, and because the supernumerary-canals defect is more penetrant, we have mainly studied the excretory canal cell as a first step towards elucidating the mechanism that controls neurite/cellular extension number. We find that the supernumerary cellular extensions form at the same time and have the same cytoskeletal organization as normal canals in HSPG mutants. We have determined that the conserved HSPG Syndecan/SDN-1 is key in this mechanism, cell-autonomously controlling cellular extension number during embryogenesis. Our genetic analysis reveals that the guidance cue UNC-6 and the guidance receptors UNC-40, UNC-5 and SAX-3, all cooperate with SDN-1 to restrict the number of cellular projections. Furthermore, we show that SDN-1 acts through two cytoskeleton modulators of the Rho-GTPases family CED-10/Rac and MIG-2/RhoG, which function in the developing cell to control cellular extension number. Our findings uncover the existence of a HSPG-regulated system ensuring the establishment of proper cell extension number during polarized cells development. Given the evolutionary conservation of the developmental mechanisms implicated, this work provides information relevant to understanding the cellular and molecular bases of the development of precise cellular morphologies in varied cell types across animals, including neurons.

849B **MIG-6/papilin mediates long-term maintenance of neuronal architecture through the regulation of extracellular matrix organization and cell signaling** Malika Nadour[1], Ivan Valette[1], Marie Biard[1], Lise Rivollet[1], Noémie Frébault[1], Philippe St-Louis[1], Andrea Thackeray[2], Maria Doitsidou[3], Claire Bénard[1,2]1Dept Biological Sciences, CERMO-FC Research Center, Université du Québec à Montréal, [2]Dept Neurobiology, University of Massachusetts Medical School, [3]Center for Discovery Brain Sciences, University of Edinburgh

After the embryonic assembly of the nervous system, neuronal circuits need to persist lifelong in the face of maturation, body movements, and aging. However, how neuronal organization is protected throughout life is not well understood. Molecular mechanisms actively maintain the architecture of the nervous system, acting with great cellular specificity. In cell-adhesion molecule mutants *sax-7/L1CAM*, distinct neuronal structures that develop normally later become disorganized. Through a genetic screen, we uncovered that loss of the extracellular protein MIG-*6*/papilin suppresses *sax-7* mutants' neuronal defects. 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*/papilin mutation, as the post-developmental depletion of collagen IV, or of its crosslinking enzyme peroxidasin, rein-

states neuronal maintenance defects in *sax-7; mig-6* mutants. Moreover, the TGF-β pathway is well known to regulate fibrosis in mammals, and here we found that not only it impacts collagen IV remodeling in the worm, but also 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. Thus, MIG-6/papilin mediates neuronal maintenance by remodeling the extracellular matrix. Understanding general principles of the maintenance of neuronal architecture and connectivity may help identify key factors influencing the onset and progression of neurodegenerative conditions.

850B **Uncovering novel neuropeptide regulators of associative behaviors in *C. elegans*** Emily J Leptich[1], Rachel N Arey[21]Neuroscience, Baylor College of Medicine, [2]Center for Precision Environmental Health and Department of Molecular and Cellular Biology, Baylor College of Medicine

Associative learning and memory behaviors are highly conserved across organisms and become disrupted in the context of aging and neurodegenerative disease, significantly compromising quality of life. Therefore, it is critical to identify mechanisms that boost learning and memory function. The nematode *C. elegans* is a fantastic model for studying this problem, given their short lifespan, invariant cell lineage, and wealth of genetic tools available. Most importantly, *C. elegans'* associative memory behavior is molecularly conserved. Learning and memory rely on synaptic plasticity to alter the strength of circuits in response to associative stimuli, which is mediated by signaling molecules such as neurotransmitters and neuropeptides. While neurotransmitters are released directly from a neuron to its post-synaptic neuron, neuropeptides can traverse longer distances and act as the wireless signaling network of the nervous system, making their mechanism of action difficult to investigate. Growing evidence indicates neuropeptides regulate learning and memory, but their roles in these behaviors are not completely defined. Our previous research in *C. elegans* shows that gain-of-function mutants in Gαq signaling (*egl-30(gf)*) have enhanced long-term associative memory (LTAM) behavior that requires neuropeptide signaling from a single sensory neuron, the AWC. However, the identities of these memory-promoting neuropeptides are unknown. Here, we sought to identify the neuropeptides and their target receptors that boost learning and memory behavior. Using an RNAi-based approach, we screened candidate AWC-expressed neuropeptides for their role in learning and memory behavior in *egl-30(gf)* animals. We found multiple neuropeptides from different families (NLPs, FLPs, and INSs) are necessary for enhanced learning and memory behavior. We also found that these peptides are required for normal learning ability in wild-type animals, highlighting our screen's ability to uncover new learning and memory regulators. In ongoing work, we are investigating target receptors of these pro-learning and memory peptides as well as the neuronal sites governing these behaviors. Overall, this research will provide insight into the molecular underpinnings of learning and memory, potentially leading to novel therapeutic targets for cognitive impairment in higher organisms.

851B **AP180 Assembly Domain Controls Synaptic Vesicle Function via Condensates and Actin Cytoskeleton** Yu Wang[1,2,3], Lanxi Wu[2], Yongming Dong[2], Aaradhya Pant[2], Lin Zhang[2], Yan Liu[2], Jihong Bai[21]School of Life Sciences, Westlake University, [2]Basic Sciences Division, Fred Hutchinson Cancer Center, [3]Fudan University

Neurotransmitter release from synaptic vesicles is a fundamental process for synaptic transmission, and changes in the size and release of these vesicles can significantly impact neuronal communication. The endocytic protein AP180/UNC-11 plays a crucial role in the maintenance of synaptic vesicle functionality. Our study revealed that the Assembly Domain (AD), an intrinsically disordered region of AP180, is critical in regulating the morphology and release of synaptic vesicles. We found that deleting the AD domain of AP180/UNC-11 resulted in enlarged vesicles and increased rates of neurotransmitter release, indicating that the AD domain regulates vesicle size and function. The AD domain forms liquid-liquid phase separation (LLPS) condensates that enrich monomeric actin and facilitate actin filament growth. Full-length AP180/UNC-11 also forms LLPS condensates, which can simultaneously recruit F-actin and liposomes. The C-terminal domain of HIPR-1 protein, which contains an actin-binding motif, can fully substitute for the AP180 AD in controlling synaptic transmission and vesicle size. However, deleting the short actin-binding region from the HIPR-1 C-terminal domain abolished its ability to substitute for AP180/UNC-11 AD. These results uncover a functional link between the AP180/UNC-11 AD and actin cytoskeleton in controlling the size of synaptic vesicles. Further, we investigated the underlying mechanism responsible for the increased rates of neurotransmitter release from enlarged vesicles at synapses lacking AP180/UNC-11 AD. Our data show that the synaptic protein Complexin has a diminished inhibitory activity on enlarged synaptic vesicles, which could account for the increased probability of neurotransmitter release at synapses lacking AP180/UNC-11 AD. At wild-type synapses, removal of Complexin led to significantly increased rates of endogenous excitatory synaptic currents (EPSCs), whereas at ΔAD synapses, EPSC rates remained unchanged with or without Complexin. Our study provides new insights into the regulation of synaptic vesicle morphology and functionality through the intrinsically disordered AD region of AP180/UNC-11, identifying a novel mechanism behind the reliability of quantal release in synaptic transmission and highlighting the crucial role of AP180/UNC-11 in maintaining proper synaptic function.

852B **Temporal Variation and Individuality in Decision-Making across Development** Smriti Bhardwaj, Shay SternFaculty of Biology, Technion

During their life, animals exhibit complex decision-making dynamics, facilitating them to elicit adaptive behavioral responses over long developmental timescales. Decision-making is mostly studied as a discrete event, during a particular life stage of an animal. However, it is still unclear how behavioral decisions are temporally organized across and within stages of development and what are the underlying processes that lead to inter-individual variation in decision-making over these long timescales. Our work employs the paradigm of exploitation vs. exploration to study temporal variations in decision-making across the entire developmental trajectory of *C. elegans.*

Monitoring multiple *C. elegans* individuals for 60 hours (from egg hatching to adulthood), during the decision-making paradigm reveals unique temporal patterns of decision-making and performance with individual differences. We found that when confronted with different concentrations of bacteria, individual worms mostly chose a high concentration of *E. coli* OP50 right after hatching and decide to continue feeding on it until adulthood. However, individuals that chose low concentration immediately after hatching correct their initial decision during early larval stages to secure the nutritional requirements for the future. In addition, we found that when embryos are allowed to hatch on low concentration food, worms can sense the high concentration and decide to leave the existing food patch even before it is completely exhausted. We further found neuromodulatory effects on decision-making dynamics across development that were sensitive to the external sensory cues.

Interestingly, our results also show differences in the time points of decisions among individuals from an isogenic population, indicating their unique individual preferences. These results suggest that exploitation vs. exploration decisions in *C. elegans* depend on extrinsic factors (e.g. competitive sensory cues) and intrinsic factors (e.g. innate motivation to explore for a better food source). Our work also implies specific developmental time points for making a food-related decision and reflects upon the timely manifestation of foraging algorithms.

853B **Neuromodulation of sexually dimorphic perceptual behaviors** Sonu Peedikayil Kurien[1], Rizwanul Haque[2], Asaf Gat[2], Meital Oren-Suissa[21]Weizmann Institute of Science, [2]Brain Sciences, Weizmann Institute of Science

Sexually dimorphic traits, an emergent property of sexual selection, govern the continuing reproductive success of the species. Shared environmental stimuli, thus, have to be processed by sexually dimorphic systems, leading to critical integration of internal and external cues to generate efficient decision-making strategies, a key component of learning behavior. However, the underlying mechanisms regulating such complex processes, including influences by the genetic sex, are still being explored.

To study whether learning is sexually dimorphic, we harnessed the well-established aversive conditioning paradigm that uses the pathogenic bacterium *Pseudomonas aeruginosa* (PA14). *C. elegans* hermaphrodites are initially attracted but soon learn to avoid PA14 after several hours of exposure (Zhang et al 2005). We found that males fail to learn and retain their naïve preference. This lack of avoidance was not due to dimorphic susceptibility, as PA14 exposure elicited similar *irg-1* mediated immune activation in both sexes. To identify the tissues regulating the dimorphic learning behavior, tissue specific sex-reversal experiments were performed. While hermaphroditic learning is governed primarily by the nervous system, male learning is modulated by distinct cross-talk of the nervous system and the gut. This is paralleled also at the circuit level, where calcium imaging indicated dimorphic information processing within the axonal regions of the interneuron AIY. To uncover the molecular entities responsible, we performed transcriptomic profiling of naïve and trained animals. While the overall transcriptomic landscapes were similar, we observed an underlying dimorphism in neuromodulation. Congruently, our behavioral screens unmasked the involvement of several neuropeptides and a neuropeptide receptor, the NPY receptor homologue *npr-5*, in switching male learning behavior – mutant males showed enhanced learning. Furthermore, changes in the endogenous levels of *npr-5*, post-training, complimented the transcriptomic data, while also shedding light on its possible sensory modulation. Likewise, calcium imaging of sensory neurons in *npr-5* mutants revealed changes in neuronal firing patterns, corroborating the modulation of perception by NPR-5.

Taken together, our results suggest that shared environmental stimuli trigger a complex decision-making process that spans multiple tissues, inputs, outputs and neuromodulators, all of which is superintended by the sexual identity.

854B **Ubiquitin Ligase Activity Inhibits CDK5 to Control Axon Termination** Muriel Desbois[1], Karla Opperman[1], Jonathan Amezquita[1,2], Gabriel Gaglio[3], Oliver Crawley[4], Nelson A. Ayala[1,5], Brock Grill[1,2,6,71]Center for Integrative Brain Research, Seattle Children's Research Institute, [2]Molecular and Cellular Biology, University of Washington, [3]Department of Neuroscience, The Scripps Research Institute, [4]Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, [5]Department of Pharmacology, University of Washington School of Medicine, [6]Pharmacology, University of Washington, [7]Department of Pediatrics, University of Washington School of Medicine

The Cdk5 kinase plays prominent roles in nervous system development, plasticity, behavior and disease. It also has important,

non-neuronal functions in cancer, the immune system and insulin secretion. At present, we do not fully understand negative regulatory mechanisms that restrict Cdk5. Here, we use Caenorhabditis elegans to show that CDK-5 is inhibited by the RPM-1/FSN-1 ubiquitin ligase complex. This atypical RING ubiquitin ligase is conserved from C. elegans through mammals. Our finding originated from unbiased, in vivo affinity purification proteomics, which identified CDK-5 as a putative RPM-1 substrate. CRISPR-based, native biochemistry showed that CDK-5 interacts with the RPM-1/FSN-1 ubiquitin ligase complex. A CRISPR engineered RPM-1 substrate 'trap' enriched CDK-5 binding, which was mediated by the FSN-1 substrate recognition module. To test the functional genetic relationship between the RPM-1/FSN-1 ubiquitin ligase complex and CDK-5, we evaluated axon termination in mechanosensory neurons and motor neurons. Our results indicate that RPM-1/FSN-1 ubiquitin ligase activity restricts CDK-5 to control axon termination. Collectively, these proteomic, biochemical and genetic results increase our understanding of mechanisms that restrain Cdk5 in the nervous system.

855B **The olfactory neuron AWC^{on} perceive the volatile sex pheromone, cyclohexyl acetate, in *C. elegans* male.** Yuki Togawa[1], Xuan Wan[2], Matthew Gronquist[3], Frank C Schroeder[4], Paul W Sternberg[2], Ryoji Shinya[11]School of Agriculture, Meiji University, Japan, [2]Biology and Biological Engineering, California Institute of Technology, USA, [3]Chemistry, State University of New York at Fredonia, USA, [4]Chemistry and Chemical Biology, Cornell University

Sex pheromones are critical cues for attracting mating partners for successful reproduction. Broadly, two types of sex pheromones are present within *Caenorhabditis elegans*: non-volatile and volatile. Though the non-volatile sex pheromones, the ascarosides, regulates short-range attraction in nematodes, the chemical composition and the role of volatile pheromones among nematodes had remained a long-standing mystery. We have recently identified cyclohexyl acetate (CA) as a first example of a long-range volatile sex pheromone produced by the hermaphrodites/females within the *elegans* group. However, the CA receptor in males has still remained unknown. The purpose of this study is to clarify the sensory neurons required for CA perception in *C. elegans* males. We carried out chemotaxis assays with specific *C. elegans* mutants to determine which neuron is responsible for CA perception. First, we assayed males with mutations that affect the function of CEM, AWA, and AWC. As a result, *ceh-36(ky640)* and *njls79 [ceh-36p::cz::casp3, ceh-36p::casp3::nz, ges-1p::GFP](X)* mutant males ,which lack the function of AWC, were almost completely defective in their response to CA. Next, to determine whether both or only one of the two types of AWC neurons is required for CA reception, we performed chemotaxis assays with 2-AWC^{on} and 2-AWC^{off} mutants. As a result, the mutant *nsy-4(ky616)* males, which shows a 2-AWC^{off} phenotype, exhibited significantly less attraction ($p < 0.0001$, Dunnett's multiple comparison test), whereas the mutants *nsy-1(ky397)* and *nsy-1(ok593)*, having 2-AWC^{on} neurons, were attracted to CA at the same level as WT males, having AWC^{on} and AWC^{off} neurons. To visualize the response of AWC^{on} neurons triggered by CA, we used the calcium indicator GCaMP6s for real-time visualization of sensory-neuron excitation by monitoring the influx of calcium ions upon exposure to the chemical signal. The male AWC^{on}-neuron GFP intensity reduced immediately after CA stimulation and rapidly decreased within the observation window of 2.5 min. CA is thus sensed by male AWC^{on} neurons. These findings offer a starting point to unravel the underlying mechanisms of volatile sex pheromone communication, especially pheromone perception, among nematodes.

856B **The mind of a dauer: EM reconstruction of neuron wiring** Hyunsoo Yim[1], Daniel Taehyun Choe[1], Junhwan Alexander Bae[2], Hae-Mook Kang[2], Ken C.Q. Nguyen[3], Myung-kyu H Choi[1], Soungyub Ahn[1], Sang-kyu Bahn[4], Heeseung Yang[1], David H. Hall[3], Jinseop S. Kim[5], Junho Lee[11]Department of Biological Sciences, Seoul National University, [2]Research Institute of Basic Sciences, Seoul National University, [3]Department of Neuroscience, Albert Einstein College of Medicine, [4]Neural Circuits Research Group, Korea Brain Research Institute, [5]Department of Biological Sciences, Sungkyunkwan University

Dauer is an alternative developmental stage of *Caenorhabditis elegans*, which is induced by harsh environmental conditions. Since the dauer larvae show quite different physiological properties and behavioral patterns from non-dauers, it is strongly expected that the neural circuit of the dauer stage may have undergone neuronal remodeling. In this study, we acquired serial sections of electron micrographic (EM) images to reconstruct the whole nervous system of a dauer. Image volumes were densely reconstructed to reveal the nervous system of a dauer, and numerous contact changes and morphological changes were newly discovered in the dauer axons which had not been previously reported. Deep-learning-based automatic synapse detection was developed to find synapses. Comparing the connectomes between non-dauer stages, dauer connectome shows big proportion of large unique connections, which means it is qualitatively different from non-dauer stages. These connection changes of dauers are mutually associated with morphological changes, and some of them also drive functional changes. These findings will contribute to the understanding of neuronal plasticity and adaptation to the environment of the organism.

857B **FLP-15 through the receptor NPR-3 regulates reversal behavior during foraging in *C. elegans*** Umer Saleem Bhat[1,2], Siju Surendan[3], Kavita Babu[31]Biological Science, Indian Institute of science education and research, Mohali, [2]Centre for Neuroscience, Indian Institute of Science, [3]Centre for neuoscience, Indian Institute of Science

Foraging for food in *C. elegans* is an amalgam of different types of locomotory movements including forward crawls, turns and

reversals. When *C. elegans* are transferred from well-fed conditions to an off-food plate, they explore the arena in a localised manner with a combination of reorientations (reversals and omega turns) executed frequently. However, the cumulative frequency of these reorientations decreases temporally in off-food conditions to release the local search and ensure global search of the arena. Defects in reorientations and/or the body wave parameters like the amplitude of sinusoidal waves result in inefficient exploration of environment. This exploration, a sustained behavior, is reported to be mediated by chemosensory and mechanosensory neurons, but the mechanism is poorly understood. Therefore, in this context, the non-wired neuropeptidergic circuit that is known to regulate sustained behaviors by virtue of its characteristic signaling mechanism, is an interesting pathway to investigate.

We screened through genetic mutants of neuropeptides for defects in local and global search behaviors. So far, from our screen, we have found that neuropeptides FLP-8, FLP-15, FLP-17, NLP-49, FLP-6, INS-3, and PDF-1 regulate the reversal frequency. Interestingly we found that FLP-15 regulates the frequency and length of reversals during both local and global search. We further observed that FLP-15, is expressed in a subset of head neurons and functions through the G-protein-coupled receptor, NPR-3, to regulate foraging behavior. Our results show that the mutants in neuropeptide *flp-15* and receptor *npr-3* show 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, the reversal frequency is significantly higher when compared to wild-type animals in global search. In an interesting observation, we also found that FLP-15, possibly through NPR-3, also regulates amplitude of the body bends. Our experiments show that *flp-15* and *npr-3* mutants show a significant increase in the amplitude of the body bends during sinusoidal movement, when compared to wild- type control animals and therefore are unable to explore their surroundings effectively during foraging. Our ongoing genetic and physiological manipulation experiments will help us in elucidating the underlying functional circuitry through which FLP-15 and NPR-3 allow normal locomotion in *C. elegans*

858B **Studying the role of asymmetric neuronal connectivity in touch sensation of *Caenorhabditis elegans*** Varun S Birari, Ithai Rabinowitch Department of Medical Neurobiology, The Institute for Medical Research Israel-Canada (IMRIC), The Hebrew University of Jerusalem

In many respects the brain is bilaterally symmetrical. However, multiple structural, functional, and behavioral asymmetries occur within the underlying overall symmetry. Such asymmetries may enable compartmentalization of function, parallel processing of information, and extended specialization. Yet, many aspects of brain asymmetry require deeper investigation, such as, the role of neuronal connectivity in the emergence of functional asymmetries, the flow of information in a lateralized brain, the roles of structural and functional brain asymmetries in the generation of various behaviors. The completely mapped neuronal connectivity, tractable genetics, and transparent body of *C. elegans* offer considerable advantages for studying structure-function relations in the nervous system. Our theoretical analysis of the connectomes showed that the asymmetry in the neuronal connectivity of the bilateral neurons is a consistent feature of *C. elegans* connectomes. To understand the functional significance of asymmetric connectivity, we focused on the gentle-touch sensing neurons, ALM (ALML and ALMR), by looking at their synaptic connectivity, recording their calcium dynamics and examining the presence of laterality in behaviors related to the ALMs. We found pronounced spontaneous calcium activity in ALMs, which unlike touch-evoked calcium activity was bilaterally asymmetrical. We further discovered that the source of the spontaneous calcium activity is not touch sensation (*mec-4*) nor chemical (*unc-13*) or neuropeptide signaling (*unc-31*) between the neurons, as the spontaneous calcium activity was observed in all the respective mutants, although with an altered pattern. When looking at the behaviors underlying ALMs, we found no apparent laterality in the escape response or the habituation response to gentle touch, but we have evidence suggesting the presence of laterality in off-food spontaneous reversals that are regulated at least in part by the touch neurons. Currently, we are focusing on the effects of symmetrizing the neuronal connectivity of ALMs on spontaneous calcium activity and the worm behaviors. Our findings distinguish between robust functional touch neuron symmetry when conveying escape responses, but at the same time functional asymmetry when regulating more subtle behaviors.

859B **Methionine analogue-based cell-specific proteomics and interactomics in C. elegans** QIAO RAN[1], Siyue HUANG[2], Xiang David LI[2], Chaogu ZHENG[11]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 physical isolation of neurons often leads to the loss of proteins in dendrites and axons. Moreover, proteins work in complexes, and currently there is no robust method to study protein-protein interaction in specific cells.

To close the technology gap, we developed Methionine Analogue-based Cell-Specific Proteomics and Interactomics (MACSP and MACSI) methods to profile the proteomes and interactomes of cells of interests 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. By fusing HA tags to endogenous chaperone (e.g., *hsp-90*, *hsp-70*, *hsp-110*, etc.) loci and using UV-induced photo-crosslinking and double-enrichment with both the bio-orthogonal tag and HA tag, we also demonstrated the profiling of chaperone interactomes in specific tissues. These newly developed chemical biology tools may be useful for understanding cell/tissue-specific protein expression and protein-protein interaction in *C. elegans*.

860B **Modifying TDP-43 Toxicity** Lale Gungordu, Mandy Koopman, Renée I Seinstra, Rene Wardenaar, Victor Guryev, Ellen A.A. Nollen European Research Institute for the Biology of Ageing

Protein toxicity is thought to underlie several, yet incurable, age-related neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). TDP-43 aggregation is the major pathological hallmark of ALS and present in 97% of all cases, suggesting that TDP-43 contributes to the disease mechanism. How protein toxicity triggers cell-and physical dysfunction and leads to degeneration is still not understood. In this project a *C.elegans* model for TDP-43 induced toxicity is used to study biological mechanisms that lead to disease-related phenotypes. For this aim, we carried out a whole genome RNA profiling and found that genes involved in calcium ion binding, ion channels, muscle function and post-synaptic reception were upregulated. Then we did a compound screen that targeting different ion channels in *C.elegans.* Our results showed that activation of L-type calcium channels or inhibition of calcium-activated large-conductance BK potassium channels could rescue the movement defects in TDP-43 worms.

861B **Development of individual neural computational capacities in *C. elegans*** Yuval Balshayi, Alon ZaslaverGenetics, Hebrew University of Jerusalem

Neurons typically form elaborate tree-like structures that support various computational functions. In *C. elegans*, however, most neurons possess a simple structure consisting of a few or even a single neurite. This highly restricts the computational capacity of the network, particularly given its small and compact size. We have recently shown that the *C. elegans* neural network comprises discrete clustered synaptic structures that may support local compartmentalized activities, thereby enhancing the computational power of the nervous system (Ruach *et al.* 2023).

But how and when do such synaptic clusters develop? To address these questions we analyzed the recently published eight connectomes (Witvliet *et al.* 2021) which span the different developmental stages of *C. elegans*. We show that synaptic clusters form early during development, mostly during the transition from the L1 to the L2 larval stages. This early development of the synaptic structures further underscores their important contribution to network functionality, and is nicely exemplified by the six RMD head motor neurons which show significant lateral and dorso-ventral clustered synaptic connectivity that may support robust and coordinated side-to-side head swings. Interestingly, these clustered synaptic organizations are highly variable across individual animals: while a core set of the clustered synapses is invariably shared among the animals, a significant fraction of these structures is uniquely found within individuals. This high animal-to-animal variability suggests that signals may be differentially propagated and integrated within neural networks of isogenic animals, thus significantly contributing to their idiosyncrasy. Together, formation of synaptic clusters can enhance the computational capacities of compact neural networks consisting of simply-structured neurons, and their highly variable occurrence within individuals may further explain behavioral variability among isogenic individuals.

Ruach *et al.* The synaptic organization in the *Caenorhabditis elegans* neural network suggests significant local compartmentalized computations. PNAS **120 (3)**, e2201699120 (2023).

Witvliet *et al.* Connectomes across development reveal principles of brain maturation. *Nature* **596**, 257–261 (2021)

862B **The effect of complex social environment on the collective behavior of *Caenorhabditis elegans*** Iris Bernstein[1], Narcís Font Massot[1,2], Jacob Davidson[1,2], Serena Ding[1,21]Max Planck Institute of Animal Behavior, [2]Centre for the Advanced Study of Collective Behaviour, University of Konstanz

Collective behavior is present in many biological systems. Group-level properties, such as the aggregation behavior of some *Caenorhabditis elegans* strains, emerge when individuals interact with their near neighbors. However, group compositions are complex; individuals interacting in a group can differ in their genetic composition and their behavioral characteristics. Hence,

the influence of the social environment and the ability to discriminate between kin and non-kin organisms play a central role in many biological processes, shaping the group structure and motion and how the group makes decisions. However, little is known about the mechanisms and the dynamics of these processes and how they influence the collective behavior.

C. elegans offers the opportunity to experimentally control and measure the genetic and behavioral characteristics of individuals within a group and to study the collective behavior of different group compositions. We investigate the solitary N2 and aggregating *npr-1(ad609)* mutant strains in a mixed population setting to quantitatively assess the social context-dependent collective dynamics: looking at not only the overall group motion patterns but also the motion and interaction rules at the individual level. Through our proposed work we seek to answer how differences within a population affect the behavior of the entire group and how the behavior of the worm depends on its own identity and the identity of the individuals it interacts with.

863B **The conserved kinase NEKL-4/NEK10 modulates hyperglutamylation-induced neurodegeneration** Kaiden M Power[1], Christopher Rongo[2], Maureen Barr[11]Genetics, Rutgers University, [2]Microbiology, Rutgers University

Neurodegenerative diseases present a severe and significant medical concern. The health of neurons depends in large part on mitochondrial localization and metabolic function. Microtubule-based transport is important for proper mitochondrial localization and function, and thus to neuron health. Consequently, proteins that affect microtubule stability play indirect yet important roles in the regulation of mitochondria function. A loss-of-function mutation of the deglutamylase CCP1 causes infantile-onset neurodegeneration and leads to excessive glutamylation of microtubules and defects in transport of mitochondria in neurons. CCP1 is evolutionarily conserved; its ortholog in *Caenorhabditis elegans*, *ccpp-1*, also functions in a subset of neurons where it affects microtubule stability of neuronal cilia. We performed a forward genetic screen to identify suppressors of *ccpp-1* age-related ciliary degradation. We identified a mutation in the NIMA-related kinase *nekl-4*, an ortholog of mammalian NEK10. NEK10 is associated with both cilia dysfunction and disrupted mitochondrial homeostasis in mammalian cells. We found that NEKL-4 did not play a role in regulating microtubule glutamylation and acted via an unknown mechanism.

To uncover the NEKL-4 mechanism of action and to determine the role of NEKL-4 and CCPP-1 in mitochondrial dynamics, we generated CRISPR-tagged fluorescent wild type, constituently active, and kinase dead forms of NEKL-4. Wild-type and kinase-dead NEKL-4 did not localize to cilia, but were associated with mitochondria. In *ccpp-1* mutants, kinase-dead NEKL-4 translocated to cilia and suppressed ciliary degeneration. Time lapse microscopy showed that NEKL-4 and mitochondria are co-transported, which suggests that NEKL-4 may regulate mitochondria transport in the ciliated neurons. Additionally, our imaging showed that both *nekl-4* and *ccpp-1* affect mitochondrial morphology, localization, and oxidation state in these neurons, all of which may impact cilia structure and function. Our work adds to a growing body of evidence suggesting a connection between cilia stability and mitochondrial function that, when perturbed, could instigate neuronal degeneration.

864B **Age-related neuronal changes are alleviated by dietary restriction in part through the regulation of neuronal architecture maintenance molecules** Yann A Chabi[1], Anagha Khandekar[2], Ju-Ling Liu[1], Lise Rivollet[1], Claire Bénard[1,21]Université du Québec à Montréal, [2]University of Massachusetts Chan Medical School

Cognitive decline during aging is well-known in humans, however the mechanisms by which neuronal dysfunction is triggered during aging are poorly understood. Previous studies have reported age-related morphological changes of *C. elegans* neurons (including by Pan *et al*, 2011; Tank *et al*, 2011; Toth *et al*, 2012). We have expanded this analysis with a systematic survey of age-related neuronal changes in wild-type animals and find that neuron-type specific structural alterations occur across the entire nervous system during normal aging. These changes are uncoupled from lifespan extension, as they were not delayed in several long-lived mutants, consistent with findings on healthspan studies (Bansal *et al*, 2015). In contrast, age-related neuronal alterations were robustly delayed in the genetic model for caloric restriction *eat-2* (using two mutant alleles *ad465* and *ad111*). To dissect the molecular pathways behind the neuroprotective effect of caloric restriction, we determined that the transcription factor *pha-4*/FOXA is required for maintaining neuronal organization during aging, similar to its requirement for *eat-2* mutants› long life. A potential target of *pha-4* is the gene *sax*-7, which is required for maintaining neuronal architecture in *C. elegans*. SAX-7 is homologous to the L1CAM family of cell adhesion molecules in mammals, where it functions post-developmentally to preserve cognitive abilities in adults. We find that the expression level of SAX-7 is upregulated in calorically restricted animals. Moreover, increased levels of *sax-7* or of *pha-4* were sufficient to preserve youthful aspects of neuronal organization in otherwise normally aging animals. Given the conservation between the human and *C. elegans* genomes, and in neuronal processes, the genes that protect from or promote neuronal decline in *C. elegans* will advance our knowledge of the principles underlying neuronal maintenance and aging and may provide insights into age-related neurodegenerative diseases

865B **The CREB-regulated co-transcription factor CRTC1 regulates stress-induced sleep** Aja E McDonagh[1], David M Raizen[2,3], Alexander M van der Linden[41]Biology, University of Nevada, Reno, [2]Perelman School of Medicine, University of Pennsylvania, [3]Chronobiology and Sleep Institute, University of Pennsylvania, [4]University of Nevada, Reno

Exposure to noxious conditions such as heat or UV-irradiation causes *C. elegans* to exhibit a period of quiescence called stress or sickness-induced sleep (SIS). Quiescence is also exhibited postprandially after a fast. Postprandial quiescence is mediated by the *daf-7*/TGF-beta pathway (Goetting *et al*. 2018). We previously found KIN-29, a homolog of the salt-inducible kinase SIK3, to act upstream of the sleep mediating neurons ALA and RIS activation, as a key metabolic regulator of sleep (Grubbs *et al*. 2020). To understand how KIN-29/SIK3 functions, we investigated known phosphorylation targets of SIKs, and found that the CREB-regulated transcription co-activator CRTC1 regulates SIS. *crtc-1(tm2689)* mutant animals are defective in movement quiescence but not feeding quiescence during SIS, and this phenotype can be rescued by overexpressing *crtc-1* controlled by its own promoter. Two mutant alleles of *crh-1*, the CREB homolog known to interact with CRTC1, also have reduced SIS as shown previously (Cianciulli *et al.* 2019). Our data suggests *crtc-1*, and consequently *crh-1*, act in the KIN-29 signaling pathway upstream of EGF receptor signaling in the ALA/RIS neurons to regulate SIS. Loss-of-function mutations in the histone deacetylase gene *hda-4* corrected the SIS phenotype of *crtc-1* mutants as it also does in *kin-29* mutants. Interestingly, the *crtc-1* mutant sleep phenotype is similar to the one observed for *daf-7* mutants involved in food-dependent plasticity of SIS where mutant animals have a more severe movement quiescence phenotype than feeding quiescence. A *crtc-1* mutation strongly reduced *daf-7* expression in the ASI neuron, and *crtc-1; kin-29* double mutants display a more severe defective movement quiescence phenotype than the single mutants. These findings may suggest that *crtc-1* acts at least partially in parallel to *kin-29* to modulate the *daf-7* pathway during SIS. We are currently investigating the *crtc-1* site of action. In conclusion, we identify CRTC-1 as a new SIS regulator, and our working hypothesis is that CRTC-1 may act both in KIN-29 and DAF-7 signaling to regulate SIS.

866B **Identifying suppressors of stress-induced neurodegeneration in the knock-in *sod-1 G85R* ALS model** Mika Gallati, Katherine Yanagi, Alexander Lin-Moore, Anne C. HartNeuroscience, Brown University

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. Approximately 10% of ALS cases have a known genetic origin, with 20% of those cases caused by mutations in superoxide dismutase 1 (SOD1). Despite decades of research, the underlying mechanisms of ALS are still incompletely understood. Therefore, identifying genetic modifiers of neurodegeneration in SOD1 ALS models can lend insight into the disease mechanisms. The Hart Lab has previously generated a single-copy expression model for SOD1 G85R, a patient allele leading to ALS; insertion of G85R into *C. elegans sod-1* causes glutamatergic and cholinergic neurodegeneration after exposure to oxidative stress (PMID: 30296255). With this model, we performed a forward genetic screen to identify suppressors of neurodegeneration (12,586 haploid chromosomes). Lines showing suppression were isolated, ranging from slight decreases in neuron degeneration to near-complete rescue. We are currently examining 25 of these lines to identify causal alleles. The three lines with the strongest suppression of neurodegeneration have already undergone backcrossing and sequencing to identify causal mutations of the suppression phenotype via the sibling subtraction method (PMID: 29237702). The remaining suppressor lines are under analysis. Identification of these suppressor genes will improve our understanding of the mechanisms underlying ALS and therefore potentially inform the development of future treatments.

867B **The AFD-specific glial microdomain cue, KCC-3, regulates multiple amphid neuron functions** Pralaksha Gurung, Sneha Ray, Aakanksha Singhvi Basic Sciences Division, Fred Hutchinson Cancer Center

Sensory information processing in the nervous system is primarily attributed to electrical signals passing between neurons. Glia, the other major cell-type of the nervous system, also play crucial roles in nervous system functions. They do so by providing structural support, regulating ion and neurotransmitter levels, and insulating vertebrate axons for faster propagation of electrical signals. Each glia can interact with multiple neurons simultaneously and respond differently to different circuit perturbations. It is not clear if, as these observations suggest, a single glia can uniquely interact with individual contacting neurons.

We use the *C. elegans* amphid sheath glia (AMsh) as a model to address this question. The AMsh interacts with twelve different sensory neuron receptive endings (NREs) at the animal's nose tip. Previously, we found that the glial KCC-3, a potassium-chloride co-transporter, specifically localizes to a microdomain within the AMsh apical membrane. This localization is around only the NRE of the thermosensory neuron, AFD, and KCC-3 is absent from glial contacts to the other AMsh-associated neurons. We have now defined how KCC-3 localizes to regulate AFD shape and function (Ray et al, in prep).

However, we found that even though KCC-3 does not affect the shape of any other neurons as expected, it does impact their functions. Specifically, the animals have impaired chemotaxis associated with the wing neurons AWA, AWB, and AWC. When expressed specifically in AMsh glia, KCC-3 can rescue the chemotaxis defects of AWC fully, and partially for AWA and AWB. Thus, KCC-3 regulates functions of associated AWA/B/C neurons without directly contacting them.

To address how the KCC-3 glial microdomains can regulate these distal non-contacting neurons, we are exploiting reagents we made that mislocalize KCC-3 outside its microdomain to either the basolateral or the apical membranes around distal neurons. In both these, and mutant backgrounds, we are evaluating different amphid neuron functions by both functional GCaMP imaging

and animal behavior studies. These experiments will directly address if specific microdomain localization of KCC-3 around the AFD-NRE regulates the glia's ability to modulate distal neuron functions.

868B **Behavioral and genetic evidence that habituation at different interstimulus intervals involves dissociable processes** Nikolas Kokan[1], Shawn Yee[2], Catharine Rankin[21]Neuroscience, University of British Columbia, [2]Psychology, University of British Columbia

Habituation is a simple form of learning that occurs when an organism attenuates its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. A known property of habituation is that the interstimulus interval (ISI), the time between each stimulus presentation, greatly impacts both the rate, depth, and memory of habituation. This research seeks a more detailed understanding of how ISI influences habituation and to discover genes that have an ISI specific effect on habituation. Animals habituate more slowly and to a lesser extent at long ISIs, but the response decrement will persist for longer than when the decrement was induced by short ISIs. This means that at longer ISIs habituation learning is slower, but memory of this learning is more stable. To investigate whether this is caused by different habituation processes being activated at short and long ISIs, I used our Multi-Worm Tracker to monitor the behavior of dozens of *Caenorhabditis elegans* on plates that receive mechanical taps at a set ISI. The worm's naive response to a tap is to reverse, moving backwards briefly; our Multi-Worm Tracker software generates detailed morphological and behavioral data for each worm as this reversal response habituates to repeated stimuli. As expected, my results indicate that while this reversal response habituated more slowly with longer ISIs, the memory of this habituation persists for at least 15 minutes. In contrast, I found that when the ISI was altered mid-experiment from a short ISI to a longer one, the response would often recover within a single interval (for example, 1 minute) to the asymptotic level of habituation at the longer ISI, demonstrating the transience of habituation at shorter ISIs. To further investigate the possibility of ISI specific habituation mechanisms, I have tested various mutant animals at short and long ISI looking for ISI-specific habituation defects. I have found several genes such as *avr-14*, a glutamate gated chloride channel alpha subunit, and *ogt-1*, homolog of O-GlcNAc transferase, that appear to play important roles in habituation at long ISIs but only have a small impact at short ISIs. This data provides both behavioral and genetic evidence for ISI-specific habituation processes. Next, I will determine if these genes are all acting in the same long ISI habituation pathway and where in the neural circuit this ISI-specific habituation is occurring.

869B ***C. elegans* neurons contain dynamic nuclear speckles involved in pre-mRNA splicing** Randall J Eck[1], Brandon P Henderson[2], Heather N Currey[2], Nicole F. Liachko[1,2], Rebecca L Kow[1,2], Brian C Kraemer[1,21]Department of Medicine, University of Washington, [2]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System

Nuclear speckles (NS) are phase-separated nuclear assemblies that store and modify factors involved in the regulation of mRNA transcription, splicing, maturation, and export. The RNA binding protein SON and the serine/arginine repetitive matrix protein 2 (SRRM2) are required for nuclear speckle formation in human cells. Previous research concluded *C. elegans* neurons lack NS, yet the *C. elegans* homologs of the essential NS scaffold proteins SON and SRRM2 expressed in neurons were not examined: D1037.1 and RSR-2. We generated transgenic lines overexpressing RSR-2 with an N-terminal clover tag under the *snb-1* pan-neuronal promoter. In neurons, RSR-2 forms distinct, dynamic nuclear puncta that co-localize with pre-mRNA splicing machinery. We also constructed a custom reporter to visualize cis-splicing dysfunction in neurons. The accumulation of GFP in neurons indicates incomplete cis-splicing due to the improper retention of an intronic start codon for a GFP coding sequence in the first *aex-3* intron. Using these two novel methods, we will identify genetic modifiers of neuronal NS dynamics and their impact on pre-mRNA splicing in neurons. These results will have important implications for the field of neurobiology, as NS regulation of RNA metabolism is required for proper gene expression in neurons during neurodevelopment and aging.

870B **Membrane Trafficking of hTMC1 in *C.elegans*** Rui WANG[1,2], Zhiqiang YAN[2], Chaogu ZHENG[11]School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China, [2]Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen, 518132, China

Transmembrane channel-like gene 1 (TMC1) is considered the long-sought pore-forming subunit of the mechanotransduction complex of the auditory system in the vertebrate inner ear hair cells. However, heterologous expression of TMC1 in mammalian cell lines found that TMC1 is trapped in the endoplasmic reticulum rather than being transported to the plasma membrane, where it is predicted to function, hindering the understanding of its function in mechanotransduction.

C. elegans, a genetically tractable model animal, encodes two tmc homologs (tmc-1 and tmc-2), functioning in ASH nociceptive neuron-mediated alkaline and salt chemosensation, muscle-mediated egg laying behavior, and mechanical sensation in OLQ neuron and body wall muscle. In transgenic studies, fluorescently labeled C. elegans TMC-1 and TMC-2 localize to the plasma membrane, which motivates us to use C. elegans as a model to understand the cellular trafficking of TMC proteins.

We established transgenic animals that expressed the human TMC1 in neurons and muscles and developed protocols to cul-

ture C. elegans embryonic cells. By using these tools to test whether TMC1 can be trafficked to the membrane and to study the molecular mechanisms involved in its trafficking, we found that the RFP-fused full length human TMC1 protein showed a weak expression on the C. elegans plasma membrane and colocalized with the membrane marker mCD8 fused with GFP. We will carry out non-cell-permeable immunostaining to further verify the membrane expression.

Moreover, we found that deletion of the N-terminal domain increased and stabilized the expression level of TMC1, suggesting that the stability of TMC1 and likely its membrane trafficking is negatively regulated by factors acting on its N-terminal domain. We plan to conduct forward genetic screens to identify such regulators by isolating C. elegans mutants that showed increased membrane expression of human TMC1.

Lasty, a recent structural study of the CeTMC-1 complex discovered a protein ARRD-6 co-purified with TMC-1. We will study the function of the C. elegans arrd-6. As a first step, we created arrd-6 knockout mutants through CRISPR/Cas9-mediated gene editing.

The overall goal of this project is to use C. elegans as tool to understand the membrane expression and intracellular trafficking of human TMC1 channels.

871B **MBL-1/muscleblind promotes neurite development by regulating *mec-3* alternative splicing** Ho Ming Terence Lee, Chun Yin Lau, Chaogu ZhengSchool of Biological Sciences, The University of Hong Kong

Regulation of RNA splicing plays an important role in the development of the nervous system. The Muscleblind family proteins are conserved tissue-specific RNA splicing regulators and are associated with the neuromuscular degenerative disorder known as Myotonic Dystrophy type 1. Recent studies also showed that Muscleblind proteins contribute to neuronal development in mice. We identified *mbl-1* as a regulator of neurite growth in *C. elegans* touch receptor neurons (TRNs) in a suppressor screen that searched for mutants with suppression of ectopic neurite growth induced by a gain-of-function *mec-7*/b-tubulin mutation. The loss of *mbl-1* also led to defects in normal neurite growth, although the defects were mild. Moreover, we found that MBL-1 promoted neurite growth by regulating microtubule (MT) stability and polarity. As expected, MBL-1 also modulates cargo transport in the TRNs through its regulation of MTs. To identify MBL-1 targets that are involved in the neuronal differentiation or MT functions, we performed transcriptomic analysis to identify MBL-1-regulated splicing events. In total, we identified 57 downregulated and 30 upregulated junctions in 26 and 13 genes, respectively, in *mbl-1(-)* mutants. Among these potential candidates, we found that the alternative splicing of the LIM-homeodomain transcription factor *mec-3* mediates at least part of the effects of MBL-1 in TRNs. MBL-1 promotes the generation of the *mec-3a* isoform, which has stronger activity in inducing the TRN differentiation genes, including the tubulin genes and MT-related genes. *mec-3a* isoform was switched to the shorter and less active *mec-3d* isoform, resulting in the reduction in the expression of TRN genes and hence neurite growth defects. In addition, we also performed a forward genetic screen to further identify targets that might not be identified from the transcriptomic studies by searching for mutants that suppressed the neurite growth defects in *mbl-1(-)* animals. This screen yielded four genes, all of which act in the DLK-1/p38 MAPK pathway, which are previously known to be associated with axonal regeneration in TRNs. However, we did not find any changes in the splicing pattern of genes in the DLK-1/p38 pathway in *mbl-1(-)* mutants, suggesting that they were not the direct targets of MBL-1. Overall, our studies provided mechanistic understanding for the function of MBL-1 in regulating neurite growth by identifying its downstream genes.

872B **Long-term associative memory formation in *Caenorhabditis elegans*: diving into its variation with changing environments** Monmita Bhar[1], Kamal Kishore[2], Hari Pradeep Narayanan[31]Centre for Neuroscience, Indian Institute of Science, [2]Centre for neuroscience, Indian Institute of Science, [3]Neurobiology, University of Konstanz

Communication is the key to survival. In order to survive in their native habitat, soil, *Caenorhabditis elegans* needed to learn to share and exchange information amongst themselves. Every organism shows preference towards certain factors. However, this preference can be modified through experience. Over the years, *C. elegans* has been proven to be an extremely important model system for studying behavioural plasticity.

One of the ways in which *C. elegans* exhibits learning is by forming long-term associative memory (LTAM). Previous LTAM studies have used presence or absence of food for conditioning. But, changing the feeding state of *C. elegans* in itself can affect downstream pathways overlapping with LTAM. Therefore, in this study, we use an associative learning paradigm using heat in combination with isoamyl alcohol (IAA), a native chemoattractant. This leads to *C. elegans* losing their attraction towards IAA due to negative association. This modified behaviour is retained in the form of LTAM.

C. elegans is known to communicate with each other under different conditions using various kinds of small molecules. However, we observe that *C. elegans* could possibly use some form of these small molecules to help in LTAM formation in either learning or memory consolidation as they show no sign of LTAM when they are removed from their original plates where they had been

trained, to fresh plates. This, in theory, should not have caused any difference in their memory formation capability as it does not interfere with the established training paradigm. We hypothesize that *C. elegans* use some form of a "revision molecule" that they secrete onto the plates to help them remember and form LTAM.

To understand the underlying molecular mechanism of this behaviour, we are in the process of performing RNA sequencing across different conditions in these organisms. To further address what causes their lack of memory retention when they are transferred to fresh plates in between training and testing, we will be performing mass spectrometry to identify the molecules that could be responsible for this.

873B **Stage-specific properties of the dauer connectome: A network perspective** Daniel T. Choe[1], J. Alexander Bae[2], Hyun-soo Yim[1], Ken C.Q. Nguyen[3], Sang-kyu Bahn[4], Hae mook Kang[2], David H. Hall[3], Jinseop S. Kim[4,5], Junho Lee[1,21]Department of Biological Sciences, Seoul National University, [2]The Research Institute of Basic Sciences, Seoul National University, [3]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, [4]Laboratory of Computational Neuroscience, Korea Brain Research Institute, [5]Department of Biological Sciences, Sungkyunkwan University

In unfavorable conditions, the nematode *C. elegans* enters an alternative developmental path known as the dauer stage and exhibits different behavioral patterns compared to reproductive stages. To understand the neuronal rewiring mechanisms underlying this change, we acquired a connectome for the dauer stage. In this study, we comprehensively compare the connectome of *C. elegans* in its dauer stage with all publicly available datasets from a network perspective. We first focus on individual neurons and connections before moving on to the global network properties of dauer for comparison. The weight distribution of stage-specific connections revealed distinct connections in dauer, which were confirmed by dauer-specific behavioral changes. Additionally, more intricate measures such as connection similarity and motif patterns point to the same neurons as distinctive in dauer. Although dauer is an alternative stage between L1 and L4, the global network properties are similar to those of the adult stage, including increased intra-class clustering and enhanced feedback connections. However, clustering among motor neurons is notably increased only in dauer, indicating a closer interaction among these neurons. Furthermore, sensory-to-interneuron (S-to-I) connections seem to play an important role in signal transduction uniquely in dauer. We hypothesize that these changes in network properties of the dauer connectome enhance the processing of subtle cues and exhibit prompt motor response as a survival strategy. In summary, we have identified unique neurons and connections that are unique to dauer and suggested candidates for further behavioral tests from a network perspective. The dauer connectome we acquired will offers a unique opportunity to compare two different developmental paths and identify characteristic features of each path.

874B **Neuropeptidergic circuitry underlying arousal and sensitization in *C. elegans*** Keertana Venkatesh[1], Lore Devriendt[1], Majdulin Istiban[1], Isabel Beets[1], William Schafer[1,21]Division of Animal Physiology and Neurobiology, KU Leuven, [2]Neurobiology Division, MRC Laboratory of Molecular Biology

Survival of every organism depends on its ability to modulate behavior in response to external stimuli. As a consequence, animals can switch between alternative behavioral states, one of which is arousal. A fundamental aspect of arousal is cross-modal sensitization, which primes specific brain circuits for increased vigilance following an arousing, often aversive stimulus. Although observed in most animals, from worms to humans, the molecular factors underlying sensitization and their mode of action in arousal circuits remain elusive. Neuropeptides, which often signal between neurons unconnected by synapses, play key roles in modulating such behaviors across species. The mechanisms by which such wireless neuromodulatory networks control behavior and interact with wired circuitry are less well understood. Using the well-characterized *C. elegans* nervous system, we are investigating neuropeptide genes and their roles in arousal circuits.

Earlier work has illustrated that exposure to a prior aversive mechanosensory stimulus (arousing stimulus) results in a heightened response to a subsequent aversive chemosensory stimulus (sensitization). This arousal state was seen to be dependent on an afferent neuropeptide, FLP-20, which is released from the gentle touch neurons upon mechanosensory stimulation, and its receptor FRPR-3, which is required in the RID neuron for both locomotor arousal and ASH sensitization. RID appears to exert its effects on arousal by releasing further neuropeptides, but their identity and neural mechanism of action are unknown. We are assaying mutants of several neuropeptide receptor genes expressed in the ASH chemosensory circuit to gain insight into the downstream, efferent neuropeptidergic circuitry governing sensitization. We will subsequently use optogenetic and calcium imaging tools to assess neural activity in ASH in wild-type and mutant animals in response to arousing stimuli *in vivo*, with the goal of broadening our understanding of the basic principles underlying arousal and sensitization.

875B **Expressing human epithelial Na channel subunits in *C. elegans* to model human salt taste** Laura van Vuuren[1], Ewout Hoorn[2], Gert Jansen[21]Cell Biology, Erasmus MC, [2]Erasmus MC

Human salt taste is one of the main drivers of dietary salt intake and directly correlates with blood pressure. Salt sensitivity and preference vary among people, but the underlying molecular mechanism of this variation is unknown. Many studies have shown

that the epithelial sodium channel (ENaC) is the main salt sensor involved in salt taste in rodents and humans. In the kidney, the activity of ENaC is regulated by proteases. Recently it was shown that differences in the salivary proteome correlate with salt sensitivity. We hypothesize that salivary proteases regulate ENaC open-probability and salt taste.

We use *C. elegans* as a model to study human salt taste. *C. elegans* is attracted to NaCl concentrations up to 200 mM and avoid higher NaCl concentrations. Low NaCl concentrations are mainly sensed by the ASE neurons and high concentrations by the ASH nociceptive neurons. Thus far, there are no indications that ENaC channels play a role. We aim to generate a humanized NaCl-taste worm model that expresses all three human ENaC subunits in the ASH cells. We used a two-step approach using CRISPR/Cas9 induced homology directed repair for each subunit. We first introduced a 3.7 kb *sra-6::gfp* construct in a save harbor locus on chromosome I. This strain showed proper GFP expression in the ASH neurons. Subsequently, we introduced the *SCNN1A* gene, encoding the ENaC α subunit, after the *sra-6* promoter and fused in frame with GFP. We used the same approach to express the genes of ENaC-β, -γ and -δ fused with mScarlet, tagBFP2 and GFP, respectively. In these animals we see fluorophore expression in the cell bodies of the ASH neurons. Our first analyses of animals that express human ENaC-α, β and γ showed that these animals were less attracted to salt in the NaCl-quadrant assay. In addition, we will use the drop assay. Finally, we will test the activity of salivary proteases on ENaC in this 'humanized' model system.

876B **Analysis and prediction of behavioural states during predatory and bacterial feeding in *P. pacificus*** Leonard Böger[1,2], Güniz Göze Eren[2], James W Lightfoot[2], Monika Scholz[11]Max-Planck Research Group Neural Information Flow, Max Planck Institute for the Neurobiology of Behavior – caesar, [2]Max-Planck Research Group Genetics of Behaviour, Max Planck Institute for the Neurobiology of Behavior – caesar

Within the nematode clade, a huge variety of different behavioural strategies have evolved in accordance with the different ecologies and environments associated across the diverse species. *Pristionchus pacificus*, while similar to *Caenorhabditis elegans* in many regards, has acquired several distinctive features and additional behaviours. Specifically, like *C. elegans*, *P. pacificus* can feed on bacteria, but its foraging behaviour can also encompass the predation of other nematodes. This depends on the formation of a phenotypically plastic mouth structure with a two teeth morph (eurystomatus) capable of both predation and bacterial feeding while a single tooth morph (stenostomatus) can only feed on bacteria. This feeding complexity makes *P. pacificus* a good model system to study decision-making between alternative behaviours and investigate the existence of additional behavioural states.

Here, we aim to identify the behavioural states of the eurystomatus mouth form of *P. pacificus* on bacterial food versus larval prey. Prior studies showed that the rate of pharyngeal contractions strongly differs whether the animal is feeding on larvae versus bacteria. Going beyond feeding contractions, we extended our analysis to include locomotion and head swings to define the behavioural parameters that diverge between these states. Moreover, the transitions between feeding states are stochastic and the duration of the single bouts can be long. Thus, automated prediction can facilitate the study of feeding behaviours in *P. pacificus*.

We present a supervised machine learning model that can reliably predict feeding behaviours in *P. pacificus* from tracking data, acquired by PharaGlow, a tool for tracking and analysing locomotion and feeding behaviours of moving worms from video data. The main features used for behavioural state prediction are the velocity, pumping frequency and head swings. Our model shows how animals transition between predatory and non-predatory feeding states, and how posture, motion and feeding differ between the different feeding states. This fine-grained analysis paves the way for a mechanistic understanding of state transitions in predatory nematodes.

In further studies, we will use this tool to elucidate the neuromodulatory mechanisms of feeding behaviour in *P. pacificus*. We will include investigating the influence of serotonin on feeding states, as it was previously shown to be involved in the regulation of predation and feeding in *P. pacificus*.

877B **Presynaptic cytosolic proteins can interact with membranes and subsequently recruit synaptic cell adhesion molecules.** Araven R Tiroumalechetty, Elisa Frankel, Peri T KurshanNeuroscience, Albert Einstein College of Medicine

The canonical model of synapse assembly consists of recruitment of synaptic cell adhesion molecules (sCAMs) through transsynaptic interactions followed by clustering of active zone scaffold proteins into nascent synaptic densities. However, we have found that the scaffold protein SYD-1 recruits the sCAM neurexin through PDZ interactions. Using Molecular Dynamics simulations, we show that SYD-1 is itself recruited to the membrane through C2 domain-membrane binding and validate the role of the different SYD-1 domains in synapse formation through domain deletions and fluorescent microscopy. We thus propose a novel mechanism for synaptogenesis involving SYD-1 recruiting neurexin and develop a pipeline to investigate other sCAMs that might be recruited through intracellular interactions.

878B **Head motor coordination: Exploring spatially-segregated signalling between interneurons and motor neurons** Hannah Owens[1], Michael Hendricks[2], Marie-Hélène Ouellette[21]Integrated Program in Neuroscience, McGill University, [2]Biology, McGill University

Rhythmic behaviour underlies locomotive processes such as walking, swimming, and flying across many vertebrates and invertebrates. Studying the circuits underlying these patterns has laid the groundwork for understanding how coordinated movement is generated, and how disruptions to these circuits contribute to pathophysiological conditions and motor diseases. Despite these advances, many of the molecular and cellular processes through which neural circuits produce rhythmic behaviour and coordinated locomotion remain to be determined. In *C. elegans*, the RIA interneuron encodes the dorsal-ventral position of the head through reciprocal connections with the SMD motor neurons. We hypothesize that RIA participates in modulating head bending by providing spatially-segregated excitatory and inhibitory feedback to motor neurons. When RIA function is disrupted, the sinusoidal gait of the animal is affected, and it displays specific head coordination defects. Using distinctive phenotypes and candidate gene approaches, we are investigating the molecular mechanisms underlying local signalling at reciprocal synapses within the nerve ring.

879B **Circuit and Molecular Mechanisms of an Associative Learning Task** Susana Colinas Fischer, Laura Molina-García, Lucy Lin, Arantza BarriosUCL

The ability of neural circuits to be changed by experience, optimising an organism's behaviour, is key to survival. We are dissecting the role of the neuropeptide PDF in mediating aversive olfactory learning to benzaldehyde in *C. elegans*. When benzaldehyde is paired with starvation, an aversive experience, *C. elegans'* response to benzaldehyde switches from attraction to repulsion (Lee 2010, Lin 2010). Here we show that both PDF-1 and PDF-2 mediate this form of aversive learning and seek to describe the underlying circuit.

Benzaldehyde is sensed primarily by the AWC neuron, which synapses onto first-level interneurons AIB, AIY and AIA to regulate naïve chemotaxis (Bargmann 1993, Chalasani 2007). The switch from attraction to repulsion during aversive learning has been shown to be driven by changes at the AWC-AIB synapse (Cho 2016). We find that AWC-ablated animals have a dampened response to benzaldehyde, but we still observe a switch in response from attraction to repulsion after aversive conditioning, indicating that learning can occur elsewhere in the circuit. Given that we also know that PDF is required to mediate aversive learning, we are looking to find which cells are the relevant source and target of PDF signalling in this behaviour. To find the source, we are using an intersectional Cre-Lox strategy to return physiological levels of PDF-1 in a ligand null mutant, and then removing PDF-1 from selective cells. To find the target neurons we are also using an approach that exploits the strength of the Cre-Lox system, to restore PDFR-1 function to specific cells.

We will then use calcium imaging to record the activity of the neurons we implicate in the PDF circuit for aversive learning, to see how the information flow through the circuit changes with learning, and how this is modulated by PDF. Given that associative learning is a highly conserved behaviour, understanding how this simple circuit is capable of supporting aversive learning will provide us with principles that can be applied to further understand how learning occurs in more complex nervous systems.

880B **Finding neural representations of navigation strategies using whole-brain imaging of freely moving *C. elegans* in an odor gradient** Helena Casademunt[1], Core Francisco Park[1], Albert Lin[2], Hongruo Zhang[3], Mei Zhen[4], Aravinthan D. T. Samuel[11]Department of Physics, Center for Brain Science, Harvard University, [2]Princeton Neuroscience Institute & The Center for the Physics of Biological Function, Princeton University, [3]Department of Physiology, University of Toronto, [4]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital

Many small animals use their olfactory sense as their primary source of information to navigate their environment. We study the neural basis of olfaction in *Caenorhabditis elegans*, a small nematode whose 300-neuron nervous system allows it to detect a wide range of odors and change its behavior to move towards favorable conditions using probabilistic chemotaxis strategies. To detect chemicals in the environment, these worms use large ensembles of sensory neurons and respond with distributed neuron activity in the brain. In animals immobilized inside a microfluidic chip, we found that sensory neurons respond reliably to an odor pulse while neurons in the motor circuit respond by modulating the probability of activity motifs. In freely moving animals in an odorant gradient, we find probabilistic motor responses. We record whole-brain activity of animals freely navigating in an attractive odorant gradient to elucidate how neural activity encodes gradient information and how this leads to probabilistic navigation strategies. Our experiments allow us to measure whole-brain activity, behavior, and changes in experienced odorant concentration at the same time. By finding how neural activity relates to sensory and behavioral variables, we hope to understand how the brain senses information from a complex environment and how it integrates this information with internal states to generate behavioral decisions.

881B **A Computational Pipeline for the Analysis of Social Behavior in *C. elegans* Across Development** David Scher-Arazi[1],

Eshkar Nir[21]Mathematics, Technion, Israel Institute of Technology, [2]Biology, Technion, Israel Institute of Technology

Throughout development, animals display a wide range of behaviors. These can be related to but not limited by foraging, resource acquisition, mate discovery and social interactions. In particular, social behavior is an important feature of organisms which is conserved across species and is crucial for the fitness of the individual, as well as the population. While social behavior is regularly studied at a particular life stage of the organism, the dynamics of inter-individual interactions across development are less explored. In addition, the underlying neuronal mechanisms that organize social behavior across developmental timescales are not known. To this aim, I developed a computational pipeline in order to quantify and analyze long-term social behavior across development among many pairs of *C. elegans* individuals. I built upon previous work done in my lab and developed tools to identify moments of physical interaction within well-sharing pairs for the purpose of quantifying the number and duration of interactions across development, as well as when during development did the worms spend the most time interacting. My method proved robust across all stages of development as well as the dynamic behavior available to free roaming worms in a well. Furthermore, I developed multiple analysis of this behavioral data in order to compare and contrast different populations of wildtype and mutant worms to further shed light on how different neuronal pathways can affect behavior. The development of this computational pipeline is a first step in understanding how complex social behaviors can develop in simple neuronal systems.

882B **Investigating roles for axon guidance and regeneration genes in GABAergic plasticity in *C. elegans*** Sophia M. Villiere, Kristi Zoga, Michael P. Hart University of Pennsylvania

Changes in neuronal and synaptic wiring are thought to underlie neurological variations such as autism, schizophrenia, and major depression disorder. After development, plasticity can result in remodeling of neuronal morphology and synapses that impact circuits and behavior. In *C. elegans*, DVB is a GABAergic neuron which undergoes progressive neurite outgrowth in males, altering synapses and associated behavior. We previously identified that conserved cell adhesion molecules regulate DVB neuron remodeling and plasticity. Are the molecular mechanisms which allow for neurite outgrowth during DVB plasticity in adult males shared or distinct from mechanisms of neurite outgrowth during developmental axon guidance or axon regeneration? We hypothesize that DVB plasticity in adulthood employs/ repurposes developmental and regenerative mechanistic frameworks to control neurite outgrowth and remodeling. To identify roles for these genes in DVB neurite plasticity, We monitored DVB neuronal morphology using confocal imaging at multiple timepoints. We compared DVB morphology in mutants of each candidate gene to controls during late larval development (L4) and in early adulthood (day 1 and day3 adult). We have identified genes implicated in axon regeneration (*daf-18*, *rig-6*, and *egl-30*) that contribute to DVB neuron adulthood plasticity, but have yet to identify axon guidance cues that contribute (*unc-6*, *unc-40*, *unc-129*, and *kal-1* have no DVB phenotype). We also monitored DVB associated behaviors including spicule protraction (aldicarb assay) and defecation behavior at day 1 and day 3 of adulthood to assess functional plasticity. We are continuing to screen additional genes for impact on plasticity of DVB morphology and behavior. This work provides insight into the genes and molecular mechanisms that function in GABAergic neuronal and behavioral plasticity in adulthood.

883B **NeuroPlant: An efficient chemotaxis screening platform to identify plant-made chemicals that affect *Caenorhabditis elegans*** Emily Fryer[1,2], Sujay Guha[1], Lucero E. Rogel-Hernandez[1], Theresa Logan-Garbisch[3], Hodan Farah[1], Ehsan Rezaei[1], Iris Molhoff[4], Adam Nekimken[5], Chandni Jaisinghani[1], Aleksandra Chudinova[1], Angela Xu[2], Sylvia Fechner[1], Alakananda Das[1], Shaul Druckman[6], Thomas R. Clandinin[6], Seung Y. Rhee[2], Miriam B. Goodman[11]Molecular and Cellular Physiology, Stanford University, [2]Plant Biology, Carnegie Institution for Science, [3]Molecular and Cellular Physiology, Neurosciences Graduate Program, Stanford University, [4]Biology, Stanford University, [5]Molecular and Cellular Physiology, Mechanical Engineering, Stanford University, [6]Neurobiology, Stanford University

Plants have evolved complex chemical strategies to communicate with their surroundings. Many of these small molecules (SMs) have medicinal properties, which humans exploited before the advent of modern pharmacology. Identifying new drug candidates among the myriad of plant derived SMs continues to be a strategy in drug development and requires efficient screening methods and new target identification strategies. Given their coevolutionary history with plants, we surmise that nematodes have the ability to detect many plant SMs and we hypothesize that laboratory studies of nematode behavior can identify plant SMs with therapeutic potential. With ~1453 GPCRs encoded in its genome, *C. elegans* is a rich source of molecular receptors that we aim to pair with bioactive SMs. With this goal in mind, we developed an efficient screening platform to evaluate chemotactic behavioral responses (attraction/repulsion) in *C. elegans* when exposed to SMs. The current iteration of our platform uses multiwell plates, standard liquid handling equipment, flatbed scanners, a custom data management workflow, and computer-vision based software for data analysis. With this system, we can screen 96 conditions in triplicate against a single *C. elegans* strain in 3 experimental days. We used this platform to screen a curated library of 90 plant SMs and 6 reference conditions for their

ability to attract or repel the standard laboratory strain of *C. elegans* (N2). In this way, we identified 37 SMs that evoke detectable responses in chemotaxis assays.

We will present the platform technology, workflow, and its performance along with studies of responses to the 37 chemoactive compounds in three mutant backgrounds: tax-4, osm-9, and tax-4;osm-9 double mutants. We are also evaluating the potential of leveraging the natural diversity of *C. elegans* and the genomic resources made available by the CeENDR project (Cook et al. 2016) to match SMs to their molecular receptors. As proof of concept, we used our platform to conduct chemotaxis assays with the CB4856 strain (Hawaii) against a panel of compounds known to elicit attraction or repulsion in N2. We have also extended this work to include 6 additional divergent strains curated by CeNDR to represent the genomic variation among wild *C. elegans* strains. Taken together these initial results move us closer to our goal of better understanding the molecular mechanisms of chemosensory signal transduction and the identification of SM/receptor pairs.

884B **The efficiency of synaptic vesicle exocytosis regulates the abundance of CaV2/UNC-2 voltage-gated calcium channels** Ame Xiong, Hongkyun KimRosalind Franklin University of Medicine and Science

Synaptic transmission depends on the influx of calcium ions to the presynaptic terminal. Calcium triggers the exocytosis of synaptic vesicles (SVs) to mediate neurotransmitter release. The conduit of calcium influx at the presynaptic terminal is the CaV2 voltage-gated calcium channel (VGCC). Therefore, the abundance of CaV2 channels is a key determinant of neurotransmitter release probability and shapes synaptic strength. However, little is known about how CaV2 channel abundance is regulated at the molecular level. Here, we uncover that the abundance of *C. elegans* CaV2/UNC-2 channels is negatively regulated by SV exocytosis. Gain-of-function *unc-2SL*(S240L) mutant animals, which show elevated synaptic release, have a reduced channel level. The introduction of *unc-13* and *unc-18* mutations, which impair SV exocytosis, into *unc-2SL* animals restores UNC-2SL channel levels comparable to those of wild-type UNC-2. These results indicate that the low levels of UNC-2 channels in *unc-2SL* mutants are caused by increased exocytosis. Conversely, a single mutation in the pore region, which causes a severe movement defect indistinguishable from that of *unc-2(e55)* null mutant, results in even higher levels than wild-type UNC-2 channels. These increased UNC-2 levels are reversed by the introduction of an open *unc-64*/syntaxin or loss-of-function *tom-1*/tomosyn mutation that increase SV exocytosis. To better understand the regulatory mechanism of UNC-2 channels, we performed a forward genetic screen and identified a ubiquitin ligase as a key regulator of UNC-2 channel abundance. Loss of this ubiquitin ligase function results in an increase in presynaptic wild-type UNC-2 as well as UNC-2SL channels. We propose a model in which an elevated rate of SV fusion leads to a high incidence of displacement of UNC-2 channels to the peri-active zone where endocytosis occurs. Once UNC-2 channels are displaced, they undergo ubiquitin-mediated endocytosis/degradation likely via UNC-26/synaptojanin and UNC-57/endophilin. Together, our data strongly suggest that the presynaptic terminal has an intrinsic homeostatic mechanism in which the efficiency of SV exocytosis is coupled with the abundance of CaV2 channels, thereby maintaining proper levels of synaptic strength.

885B **Increased iron promotes axon regeneration after injury** Carrie Ann Davison, Marc Hammarlund Yale University

Axon regeneration is required for neurons to restore function after injury. We are studying axon regeneration in *C. elegans* using laser axotomy of GABA neurons as an injury model. We have identified a novel and highly significant role for iron metabolism in regulating axon regeneration.

Iron levels in the nervous system must be tightly regulated for proper neuronal function. Iron in the form of iron sulfur clusters is required as a cofactor for numerous mitochondrial respiratory complexes. However, iron overload is associated with mitochondrial dysfunction, oxidative stress, neurodegenerative disease pathogenesis, and ferroptosis. We found that the cysteine desulfurase NFS-1, an enzyme required for the formation of iron sulfur clusters, is a potent inhibitor of axon regeneration. Loss of *nfs-1* strongly promotes axon regeneration in the VD/DD GABAergic motor neurons of *C. elegans*. This led us to hypothesize that increased intracellular free iron, at the expense of iron-sulfur cluster formation, allows high regeneration in the absence of *nfs-1*. We tested whether iron alone can promote regeneration. Surprisingly, supplementation with iron after injury has a dramatic effect on regeneration in wild-type worms. Nearly every axon in worms treated with excess iron fully regenerates within twenty-four hours, in contrast to fewer than 50% of axons in control animals.

We are currently exploring the cellular mechanisms that underlie the effect of iron on regeneration. Interestingly, the regeneration effect does not appear to be caused by increased oxidative stress or altered mitochondria function. Our ongoing efforts are examining iron's effects on the transcriptome, the proteome, and the neuronal cytoskeleton. Overall, our results redefine our understanding of the importance of iron for neuronal health and suggest iron and its downstream effectors as a potential therapeutic strategy for axon regeneration.

886B **"Notch-your" average *goa-1* study: sleep, insomnia, and *C. elegans*** Adam Friedberg[1], Jacqueline Cho[2], Manuel Lamela Gomez[2], Anne C Hart[11]Neuroscience, Brown University, [2]Brown University

A key characteristic of sleep is behavioral quiescence, which is observed as a cessation of movement and feeding. Behavioral quiescence is the result of coordinated changes in cellular and molecular signaling, including changes in Notch receptor and G-protein coupled receptor signaling (GPCR). In *C. elegans*, the conserved Notch signaling pathway regulates developmentally timed sleep (DTS) during lethargus. DTS is decreased when both Notch co-ligands (*osm-7* and *osm-11*) are knocked-out, while anachronistic sleep, a lethargus-like sleep behavior in adults, is produced when OSM-11 is overexpressed (Singh 2011). A gene encoding a G-alpha(o) subunit, *goa-1*, was identified in a prior unbiased forward genetic screen in *C. elegans* that found genes disrupting anachronistic sleep (Huang 2017). Interestingly, a genome wide association study in humans identified a significant association of GNAO1 with insomnia. Here, we continue investigating the role of *goa-1* in *C. elegans* sleep using CRISPR-Cas9 to knock-in orthologous GNAO1 missense mutations from human insomnia patients. Our goals are to further define the function of G-alpha(o) in *C. elegans*, understand GPCR signaling in sleep, and expand our knowledge of the mechanisms underlying insomnia.

887B **Sex specificity of neurodegeneration in *C. elegans:* the dual role of dafachronic acid** Giada Onorato[1,1,2,3], Giuseppe Guardascione[1], Giuseppina Zampi[1], Maria Paglione[1], Pamela Santonicola[1], Francesca Sola[1], Ferdinando Di Cunto[3,3,4], Adriana Maggi[5], Elia Di Schiavi[11]Institute of Biosciences and Bioresources (IBBR-CNR), [2]Department of Environmental, Biological and Pharmaceutical Science and Technologies, Università degli Studi della Campania "Luigi Vanvitelli", [3]Department of Neuroscience "Rita Levi Montalcini", University of Torino, [4]Neuroscience Institute "Cavalieri Ottolenghi", [5]Department of Pharmacological and Biomolecular Sciences, University of Milan

Oxidative stress, damage to DNA and membranes, and accumulation of damaged proteins increase with ageing, thus promoting neuronal degeneration and the occurrence of neurodegenerative diseases, which usually arise with a different incidence in the two sexes. The molecular mechanism causing this gender-specificity in neurodegenerative diseases is still elusive and its understanding is crucial to develop new and effective therapeutic strategies for differential treatments of men and women affected by neurodegenerative disease. We found that D-type motoneurons and dopaminergic sensory neurons in *C. elegans* are differentially affected by ageing in the two sexes, with aged hermaphrodites showing more morphological defects than males. On the contrary, the touch receptor neurons degenerate more in males. We confirmed this sex-specific neurodegeneration in a *C. elegans* model of Parkinson's Diseases (PD) due to overexpression of *LRRK2*^{G2019S} in dopaminergic neurons. These evidences gave us the opportunity to study why neurodegeneration and neurodegenerative disorders occur with a different incidence in the two sexes. In the PD model the higher level of degeneration of dopaminergic neurons in hermaphrodites compared to males was confirmed at the functional level. After excluding a cell-autonomous effect and the involvement of the sex-determination pathway in dopaminergic neurons degeneration, we found that cues external to neurons and originating from the female germline play a role in neurodegeneration. To discover the genetic pathway involved in the neurodegeneration modulated from the germline, we analysed loss of function alleles in two genes known to regulate ageing from the germline and found that *daf-9* and *daf-12* exhibit an opposite function in the two sexes, by acting as neuroprotective genes in males and as sensitizing ones in hermaphrodites. These results allowed us to identify the Dafachronic Acid pathway as one of the causes of the sex-specific differences in a *C. elegans* model of PD and provided new insights on the molecular events triggering neurodegeneration.

888B **RNA regulation in neurons and the germline induced by the odorant butanone** Samiha Tasnim, Antony M JoseUniversity of Maryland

Behavioral adaptation to dynamic environments is a hallmark of all animals and has been proposed to require epigenetic changes, although the underlying mechanisms are not well understood. *C. elegans* displays adaptation in response to the attractive odorant butanone such that prolonged exposure to butanone causes the worms to lose the attraction. Butanone is sensed using the AWC_{ON} sensory neuron and adaptation to it has been reported to require MUT-7, an exonuclease involved in RNA silencing, and SAEG-2, a chromatin interactor, thus implicating RNA and chromatin in mediating epigenetic changes during adaptation. However, both MUT-7 and SAEG-2 are abundant in the germline, and it is unclear whether these requirements reflect a role in the sensory neurons of the animal responding to butanone or in the germline of its parents, resulting in developmental changes that alter the response to butanone. Another possibility is that the response to butanone in neurons modulates regulators that act in the germline to cause transgenerational effects. This speculation is supported by the ability of *C. elegans* to transport double-stranded RNA (dsRNA) from neurons to the germline through the dsRNA importer SID-1. To understand the molecular processes required for adaptation to butanone and where they occur, we examined various mutants for adaptation to butanone using a choice assay that we modified to obtain a robust response with a large effect size. This assay revealed a modest adaptation defect in animals that lack SAEG-2, and a defect in butanone sensation in animals that lack MUT-7, which precludes evaluation of its role in adaptation. While SID-1 was not required for butanone sensation or adaptation, the Maelstrom-domain protein RDE-10, which promotes the amplification of secondary small RNA, was specifically required for adapting to but not

for sensing butanone. Together, these results implicate RNA-mediated and chromatin-mediated regulation in the adaptation to butanone, but additional experiments are required to determine if these epigenetic regulatory events occur within the neurons and/or are the consequence of effects within the germline.

889B **Understanding and Modeling the Integration Properties of a Neuron in *C. elegans*** Amanda C Ray, Andrew Gordus
Biology, Johns Hopkins University

Organisms integrate various inputs as they navigate their environments. At the cellular level, this occurs in neurons that integrate the inputs from their presynaptic partner. How individual neurons in the brain process this information as a function of presynaptic identity is difficult to ascertain in larger, complex organisms. With 302 neurons and a fully mapped connectome, *Caenorhabditis elegans* is well-equipped to address this problem.

Neurons with complex branching dendrites can integrate multiple inputs locally on each branch within a single neuron (*Ruach R., et al. PNAS 2023*), and can perform nonlinear computations to enhance communication between neurons if inputs are close together spatiotemporally within the same branch (*Polsky A., et al. Nat. Neuroscience 2004*). Despite most neurons lacking branching dendrites, many worm neurons exhibit presynaptic bunching at defined loci on neuronal processes. To test whether a similar phenomenon of nonlinear integration occurs in *C. elegans* neurons, we are investigating the integration properties of the AIB interneuron, which is an integration hub of many sensory and motor inputs. Using a combination of optogenetics, odor stimulation, and inherent motor activities, we find that for certain presynaptic pairs, synergistic integration rather than linear integration more accurately models the response of AIB to different combinations of presynaptic activation, and this seems tied to specific combinations of presynaptic pairs. This synergism increases the amount of information a neuron can encode, and increases the computational complexity of information encoding by the worm in general.

890B **Developmental plasticity in foraging behavior in *C. elegans*** Jorge A Luna Herrera[1], Sreeparna Pradhan[2], Michael Hendricks[11]Biology, McGill University, [2]Picower Institute for Learning and Memory, Massachusetts Institute of Technology

Early life experiences can alter adult traits in living organisms. In many instances, these changes emerge so organisms can cope with anticipated conditions correlated with the stimuli perceived during development. Nonetheless, if these adaptations do not match the future environment of these organisms, these alterations might no longer be adaptive, and could even be detrimental for them.

Previously, our research group identified that worms undergoing starvation-induced dauer arrest and recovered with an *ad libitum* regime exhibit distinct behavioral patterns during adulthood in comparison to individuals fed *ad libitum* during their whole lifetime. The occurrence of this developmental plasticity requires the expression of the ancestral allele of *glb-5*, present in the wild isolate CB4856

We are using an intersectional approach comprising 3 different strains varying in genetic background and *glb-5* allele to identify gene expression changes associated with developmental plasticity in foraging behaviour. This will allow us to obtain a better understanding of the mechanisms mediating developmental plasticity, and how early life experiences exert an effect in mature organisms.

891B **Semaphorin signaling pathway restricts neuronal regeneration** Maria belen Harreguy Alfonso[1], Zainab Tanvir[1], Esha Shah[1], Blandine Simprevil[2], Shareef Syed[1], Naomi Shah[1], Natalie Ableson[1], Ayman Mohammad[1], Tracy S Tran[3], Gal Haspel[11]Biological Sciences, New Jersey Institute of Technology, [2]City University of New York, [3]Biological Sciences, Rutgers University Newark

Signaling pathways that mediate neuronal growth cone guidance during development may also play a role in neuronal regeneration and recovery from injury. One family of signaling proteins are the semaphorins and their highly conserved receptors, the plexins. The roles of semaphorins and plexins in axon pathfinding and synapse formation during development are highly conversed across many species, from *C. elegans* to humans. In the mammalian nervous system, there are over 25 semaphorins and 9 plexins, whereas the *C. elegans* genome only encodes for 3 semaphorins and 2 plexin receptors. The transmembrane semaphorins SMP-1 and SMP-2 signal through their receptor PLX-1, while the secreted semaphorin MAB-20 signals through PLX-2.

To investigate the role of semaphorin signaling in neuroregeneration *in vivo*, we utilized *C. elegans'* natural ability to regenerate neuronal processes after injury. The small number of signaling components in *C. elegans* and our capability to precisely disconnect single neurites using femtosecond laser microsurgery make *C. elegans* an ideal model organism to address this question.

We characterized the transcriptional neuronal expression patterns of all semaphorins and plexins by co-expressing fluores-

cent proteins driven by their promoters in the NeuroPAL strain, which allows for the unambiguous identification of individual neurons. We identified the specific signaling components expressed in the motoneurons targeted for microsurgery and their surrounding tissue. Then we investigated the regrowth and reconnection of motoneuron neurites as well as the recovery of locomotion behavior following laser microsurgery in plexin- and semaphorin-knockout strains.

Our findings revealed that regrowth and reconnection were more prevalent in the absence of semaphorins-plexins signaling, consistent with their inhibitory effects in axonal growth and guidance during development. These results suggest that the secreted and membrane-bound semaphorin signaling pathways both restrict regeneration using distinct processes that likely include spatial specificity and recurrent signals. The recovery of locomotion surpassed regeneration in all genotypes, suggesting additional mechanisms for resilience.

To identify specific locations of semaphorin activity and determine its spatiotemporal response to injury, we are generating transgenic animals with fluorescently tagged secreted semaphorin (MAB-20) and animals in which a split fluorescent protein indicates the apposition of the plexin (PLX-1) with membrane-bound semaphorins (SMP-1 and 2).

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892B **Multi-omics screen identifies anti-oxidants as novel KIN-29/SIK targets in the metabolic regulation of sleep** Pearson McIntire[1], David Raizen[2,2,3], Alexander Van der Linden[11]University Nevada Reno, [2]Neurology, University of Pennsylvania, [3]Chronobiology and Sleep Institute, University of Pennsylvania

Mounting evidence suggests reciprocal interactions between sleep and metabolic tissues, however the molecular mechanisms underlying these interactions are unclear. Previously, we demonstrated that KIN-29, a homolog of the AMPK-related salt-inducible kinase 3 (SIK3), is a metabolic regulator of sleep (Grubbs *et al.*, 2020). Mutants lacking *kin-29* function exhibit high fat stores and reduced sleep (both stress-induced and developmentally-timed sleep). Genetically liberating their high fat stores partially corrects both the *kin-29* mutant's adipose and sleepless phenotypes. To identify novel targets and pathways mediated by *kin-29* activity in the metabolic regulation of sleep, we performed both proteomic and transcriptomic comparisons of wild-type and *kin-29* mutant adult worms (*oy38* null animals). Our approach identified 1847 genes with differentially expressed transcripts, of which 1796 were upregulated and 51 were downregulated in *kin-29(oy38)* mutants compared to wild-type (>1.5 log fold change & adjusted $P < 0.05$). Using the same parameters, we identified 65 differentially expressed proteins, among which 50 proteins were increased in abundance and 15 proteins had a decrease in abundance in *kin-29(oy38)* mutants compared to wild-type. Many of the proteins and transcripts of increased abundance encoded antioxidant genes involved in the mitigation of reactive oxygen species (ROS) such as superoxide dismutases, catalases, and glutathione S-transferases. Therefore, we compared ROS levels of wild-type and *kin-29(oy38)* mutant lysates showing that *kin-29(oy38)* mutants have decreased ROS levels relative to wild-type animals. Additionally, *kin-29(oy38)* mutants exhibit resistance to the oxidative stressor paraquat suggesting these upregulated anti-oxidant genes have physiological relevance. Currently, we are investigating the role of ROS in the metabolic regulation of sleep mediated by KIN-29. Our findings suggest that antioxidant pathways are novel candidate signaling targets of KIN-29.

893B **Developmentally regulated shedding of sensory cilia** Rachel Swope[1,2], Irina Kolotuev[3], Maxwell Heiman[1,21]Harvard Medical School, [2]Boston Children's Hospital, [3]University of Lausanne

Cilia are conserved organelles that sense diverse extracellular cues. In *C. elegans*, ciliated sensory neurons extend a single unbranched dendrite to the nose that terminates in a nonmotile sensory cilium. While some of these cilia are directly exposed to the external environment and sense chemical cues, many of them are embedded within specializations of the cuticle extracellular matrix (ECM) and use these cuticle attachments to sense mechanical force. This poses a problem: *C. elegans* shed and remake the cuticle at four developmental molts, raising the question of what happens to cuticle-embedded cilia during molting. Using fluorescence microscopy and array tomography scanning electron microscopy (AT-SEM), we found that large fragments of these cilia are cut off and shed with the old cuticle, and then rapidly re-grow. We developed a strategy to reproducibly stage and image the cilia of animals at the L4/adult molt, and observed shedding of all cuticle-embedded cilia in the head, including those of the cephalic (CEP), outer labial (OL), and inner labial (IL) sense organs. By contrast, we did not observe shedding of cilia in the major sense organ, the amphid; these cilia are not embedded in the cuticle. Interestingly, shedding of cilia and flagella has been well-documented in protists such as the single-celled algae *Chlamydomonas.* Examples of cilia shedding in mammals have also been reported, including shedding and regrowth of motile cilia in the oviduct epithelium during the estrous cycle and the circadian shedding of the distal portions of photoreceptor outer segments in the retina. Cilia shedding in *C. elegans* suggests either a cell-autonomous severing pathway possibly conserved with protists or non-cell-autonomous 'pinching off' by neighboring glia, reminiscent of the pruning of dendritic spines in vertebrates. In principle, developmentally regulated cilia shedding

could provide a powerful mechanism to completely reset the structural components or sensory receptors of the cilium at each life stage.

894B **The tubulin acetyltransferase *atat-2* genetically interacts with *klp-4* in *C. elegans*** Michael Webb, Claire Reist, Jay PieczynskiRollins College

Kinesins are a class of mostly microtubule plus end directed motor proteins that carry cargoes from the interior of cells to the periphery. *C. elegans* have long been a model system to study the role of kinesin motor proteins *in vivo* due the direct impact that kinesin activity has on neuronal function. We previously described an allele of the worm kinesin, *klp-4(ok3537),* that displays properties of a constitutively active motor. As a result, these KLP-4 mutants have defects in cholinergic signaling. Considering that neuronal development and function is predicated on kinesin movement down stable microtubule tracks, we next decided to investigate the role of microtubule stability in KLP-4 mediated cholinergic signaling. Using a pharmacological approach, were able to show that artificially stabilizing microtubules or preventing their assembly impacts the *klp-4(ok3537)* cholinergic signaling phenotype. *In vivo*, microtubules are stabilized by acetylation of alpha tubulin. *C. elegans* contain two alpha tubulin acetyltransferases, *mec-17* and *atat-2.* Cholinergic hypersensitivity can be rescued when *klp-4(ok3537)*, *atat-2* null mutants, but not *klp-4(ok3537), mec-17* null mutants suggesting a specific genetic interaction between *klp-4* and *atat-2.* These results demonstrate a functional interplay between microtubule stability and kinesin mediated trafficking in neuronal development and maintenance.

895B **Multiple roles for innexins in *C. elegans* thermo-nociceptive plasticity** Parvathi Sushama Gopinath[1], Dominique S Glauser[21]Department of Biology, University of Fribourg, [2]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. Connexins are among the proteins recently shown to control nociceptive signaling and pain conditions in vertebrates. Connexins form gap junctions between cells, but can also form channels and hemichannels, which can release molecules such as neurotransmitters. Studying the roles of connexins in the nociceptive pathways of vertebrates *in vivo* is very challenging. With powerful neuro-genetic tools and reduced ethical concerns, *C. elegans* represents a valuable alternative model. The *C. elegans* genome encodes about 25 innexin genes, which are structurally and functionally related to vertebrate connexins. Innexins have known roles in embryonic and larval development, fertility, neuronal fate determination, neurite development, and establishing locomotor circuit. Several recent studies have also started to highlight their role in the sensory system, including for mechanosensation, thermotaxis and as modulators of the sensitivity to aversive chemicals. Here, we performed a reverse genetic screen to learn more about the role of *C. elegans* innexins in controlling thermal nociception and its plasticity. We tested mutants for 19 innexin genes obtained from the CGC using a computer-assisted avoidance behavior analysis pipeline previously established in our lab. We quantified the worm spontaneous reversal rate, the thermal sensitivity of heat-evoked reversals (based on dose-response curves) and nociceptive plasticity by evaluating the impact of repeated noxious stimuli over 40 min. We observed diverse phenotypes among different innexin mutants, suggesting that specific behavioral aspects are controlled by specific innexins in *C. elegans*. We intend to work more on these innexins to identify the neurons and circuits that are responsible for these phenotypes in *C. elegans* and better understand the role of gap junction proteins as a whole in controlling nociception.

896B **Endogenous Tau levels predict pattern of APOE4-induced neurodegeneration in *C. elegans*** Andy Cardona, Annika Agnihotri, Jon Pierce Department of Neuroscience; Center for Learning and Memory, Waggoner Center for Alcohol and Addiction Research; Institute of Cell and Molecular Biology, University of Texas at Austin, USA

Alzheimer's Disease (AD) displays a characteristic pattern of cell death across the brain, explaining the loss of corresponding faculties like memory and executive function. The basis for this pattern of degeneration remains a mystery; however, there are some clues. The chief genetic risk factor for AD, the e4 variant of the APOE gene (APOE4), hastens disease onset and modulates AD symptom severity. Also, the overall pattern of degeneration in AD correlates with the aggregation of the microtubule associated protein tau (MAPT/Tau). However, it is largely unknown how APOE4 and Tau together contribute to the spatiotemporal pattern of cell death in AD, as most in vivo models do not feature widespread degeneration as in humans.

To address this, our lab previously generated a model of neurodegeneration in C. elegans, wherein human APOE4 is pan-neuronally expressed. We observed that the egg laying neurons, HSNs, die in middle adulthood while other neurons appear superficially healthy and functional. To determine if our APOE4 model exhibited wider spread degeneration mimicking the human condition, we probed three additional behaviors and inspected morphology of their requisite neurons. We hypothesized that endogenous levels of the sole worm ortholog of Tau, ptl-1, predicts which neurons become dysfunctional and their respective ages of deficit onset. Interestingly, we found that both the onset age and severity of behavioral deficits tend to correlate with the expression level of ptl-1 in behavior-associated neuronal subcircuits. To test if ptl-1 contributes causally to degeneration, we

crossed a deletion allele of ptl-1 into an APOE4 background. We found that deletion of ptl-1 suppressed all behavioral deficits induced by APOE4. We next asked whether PTL-1 might spread between neurons. To test this, we selectively knocked down ptl-1 in the six gentle touch neurons, which have among the highest ptl-1 levels in the worm nervous system. We observed that APOE4-induced dysfunction was suppressed in the HSN neurons. These preliminary KD experiments suggest that neurotoxic PTL-1 may spread across the nervous system down a gradient intra- or extra-synaptically. Altogether, our results suggest that ptl-1 represents a critical vulnerability factor in our APOE4 model of patterned degeneration. Continued study of cell vulnerability in our model may inform cellular molecular mechanisms that underlie how APOE4 and Tau contribute to patterned neurodegeneration in AD and assist in developing of neuroprotective therapeutics.

897B **Mechanosensory and Proprioceptive Neurons Mediate the Precipice Response in *Caenorhabditis elegans*** Savannah McCoy[1], Robin Mitchell[2], Hannah Powell[1], Shuyu Zhang[2], Will Pulice[2], Diana Pattillos[2], Katja Hodnett[2], Jared Young[11]Mills College at Northeastern University, [2]Mills College

The precipice response in *C. elegans* is a behavior wherein a worm will rapidly reverse upon the first instance of its head extending past a 90 degree edge. This behavior was mentioned briefly in a Wormbook section on Mechanosensation Behavior written by Martin Chalfie (Hart, 2006), but until recently the precipice response was not characterized or investigated in detail. In previous work, we developed a unique assay to observe and quantify the behavior of worms as they encounter cliffs. This assay was used to characterize the behavior and conduct experiments with mechanosensory mutants. Those experiments suggested that mechanosensation plays a role in this behavior (Mitchell et al., 2021).

We report new research on the mechanisms underlying the precipice response, with a particular focus on the specific neurons involved. Using a number of mutant strains and transgenic lines, we isolated the roles of several mechanosensory neurons in the precipice response, finding that the ALM, AVM, PVD, and FLP neurons are required for normal rates of precipice response. We also investigated whether force vectors influenced this behavior by comparing the rates of precipice response in worms assayed perpendicular to the gravity vector to worms aligned with the same plane as the gravity vector. We found that force vectors do not influence the precipice response, as when N2 worms encounter an edge while on the top of an agar chunk they do not display significantly different rates of precipice response than when they are assayed on the side of a chunk. We also examined whether the precipice response was a response to a novel stimulus. Most *C. elegans* raised in a laboratory setting are reared on flat agar plates, where they do not experience the sensation of a loss of substrate from underneath their heads. We wanted to investigate whether the precipice response was in part due to a worm reacting to a novel stimulus. To test this, we reared N2 worms in complex 3D environments, where they experienced 90 degree edges often throughout their development. We then compared the precipice response rates of those worms to worms reared on flat agar plates. We found that the precipice response is not influenced by a worm's experience in regards to 90 degree edges, as worms reared in complex 3D environments showed no significant difference in rates of precipice response from worms reared on flat plates. We are also testing the Hawaiian wild type strain, which has a much greater propensity to burrow in agar than N2, to see whether the Hawaiian worms behave differently in the precipice response assay.

Hart, Anne C., ed. Behavior (July 3, 2006), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.87.1

Mitchell RM, Pattillos DS, Zhang S, Young JJ. Deficient mechanosensation in *mec-3* decreases precipice response in C. elegans. MicroPubl Biol. (2021)

898B **Microcystin-LR is neurotoxic via binding the PP2A subunit C homologous protein LET-92 in *C.elegans* neuron** Chunhua Zhan Huazhong University of Science and Technology

The pollution of microcystins-LR (MC-LR) has become a growing public health concern. MC-LR can go through the cerebral blood barrier then cause neurotoxicity, however, the mechanisms underlying the negative effects of MC-LR on the peripheral nervous system function remain largely unknown. *Caenorhabditis elegans* has served as an ideal model for the study of neurotoxic mechanism due to its well understood neural network and capability.

After exposing to the environmental pollution concentration, MC-LR accumulated in the head sensory neuron cilia of nematodes rather than in the intestine where had most uptake amount of MC-LR from the environment. We determined the negative effect of MC-LR on the sensory neurons through the behavior analysis like repel odor response, chemotaxis, mechanical stimulation, locomotion and local search ability and short-wavelength light response. We found that it had significantly defection of the worm to response these stimuli after exposure to MC-LR. Moreover, MC-LR can cause redundant beading and ectopic branches in the nociception sensory neuron ASH and mechanical stimulus sensory neuron ALM. Besides, MC-LR also significantly reduced the calcium response of ASH and AWC neurons when exposure to the corresponding odors.

Further we want to know the molecular mechanism of MC-LR induced sensory neuron toxicity. PP2A is an intracellular phosphokinase that regulates important cellular processes. In *C. elegans*, LET-92 is a homologous protein of the mammal C subunit of PP2A. We found that MC-LR reduced PP2A activity by binding to LET-92. Moreover, MC-LR also reduced LET-92 expression level and nuclear localization ability in neurons. Based on the binding sites of MC-LR in mammal C subunit, we screened four key amino acids that bind to MC-LR in LET-92 via CRISPRE knock-in mutation. We were surprised to find that after mutated the L252 and C278 amino acid sites, MC-LR no longer binds to LET-92, but without affecting PP2A function.

Current studies found that PP2A can inhibit the JNK-1(c-Jun N-terminal kinase) signaling pathway by dephosphorylation, thereby regulate the morphology of neurons in mammal. In *C. elegans*, the JNK family includes JNK-1 and KGB-1. We found that after exposure to MC-LR, the neurons had a significant increase in the phosphorylation level of JNK-1, which indicated the function of PP2A got inhibited. Next, RNAi *jnk-1* and *kgb-1* in neurons we can rescue the phenotypes of sensory behavioral and morphology defects caused by MC-LR.

In summary, we uncovered the mechanism of MC-LR toxicity to sensory neurons from the animal behavior, neuron cellular activity and molecular signaling pathway. More interestingly, we found that the mutations of the two key MC-LR binding sites L252 and C278 in LET-92 erased the binding ability but did not affect PP2A activity, providing the ideas of developing a pharmacological detoxification for MC-LR exposure.

899B **Microtubule Organization in glia vs neurons** Liu He, Busra Kuloğlu, Lotte van Beem, Martin Harterink Utrecht University

Neurons heavily rely on the microtubule cytoskeleton to transport cargoes over long distances. Differences in microtubule organizations between axons and dendrites allow for selective transport into either neurite to set up neuronal polarity. Previously, we identified NOCA-2 (NINEIN) to be an important factor to organize dendritic microtubules with their minus-end outwards; NOCA-2 localizes to the distal dendrite, where it recruits the microtubule nucleation factor ɣ-tubulin (He et al., 2022). Interestingly, we observed that NOCA-2 is also abundantly localized to distal segments of the sheath Glia cells surrounding the sensory neurons in the head and tail. Considering the extended morphology of glia cells and the distal localization of NOCA-2, we set out to investigate if glia cells share a similar microtubule organization and how this may contribute to glia morphology and function.

900B **Controlling the progression of neurodegenerative diseases: A translational approach to screening small molecule inhibitors targeting aggregate formation** Tosca Bink[1], Raya Sadighi[2], Tasnim H. Alhow[1], Majorie M.B.M. van Duursen[1], Anouk M. Rijs[2], Samantha Hughes[11]A-LIFE Amsterdam Institute for Life and Environment, Section Environmental Health and Toxicology, Vrije Universiteit Amsterdam, [2]Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam

With the increase in the average age of the world's population, there is a concurrent increase in the occurrence of age-related diseases such as Parkinson's and Alzheimer's Disease. These are diseases for which no treatment exists, only symptom-relieving medication. It is expected that a successful treatment will increase when inhibitors are developed that work on earlier stages of the disease, when the neuron damage is still limited. However, to understand how the small molecule inhibitors impede the aggregation process that leads to disease, requires knowledge of the underlying protein aggregation mechanism where soluble proteins transform into insoluble fibrils.

C. elegans is an attractive system with which to study the impact of novel compounds on the aggregation process and onset of disease. We have therefore developed a high-throughput platform using the nematode Parkinson's Disease (PD) strain to screen and evaluate potential small molecule inhibitors that slow the onset and progression of the disease. The focus is on observing overall toxicity but also the impact of the molecules on aggregation and mobility, two key hallmarks of PD. Currently, are exploring the impact of these novel therapeutic compounds on the ability of the aggregates to form. To achieve this goal, an extraction method has recently been developed to isolate native α-synuclein from the nematode, which is subsequently analysed using ion mobility spectrometry–mass spectrometry. This analytical method allows us to probe the interaction and mode of action of the inhibitors on the developing aggregates. As no cure is currently available for any neurodegenerative disease, it is important that new drugs and drug targets are identified. Our platform provides a solution for the need to have an ethical cost-effective test to yield data at the whole-organism level as part of the drug development process, ultimately providing a system with which to examine and improve therapeutics to ensure people living with neurodegenerative disease remain cognisant and independent for longer.

901B **The Sole *Caenorhabditis elegans* Inositol Monophosphatase Homolog Functions in the ALA Neuron to Modulate Stress Induced Sleep** Manuel Alvarez, Han Wang Department of Integrative Biology, University of Wisconsin- Madison

Epidermal Growth Factor (EGF) promotes sleep in many organisms including humans and nematodes. In C. elegans, stress in-

duced sleep (SIS) occurs when the worms are treated with various cellular stressors such as UV light, infection, heat/cold shock, and injury. These cellular stressors activate EGF signaling primarily in the ALA neuron, which leads to the release of several sleep-promoting neuropeptides from ALA to initiate SIS. However, the genetic pathway within ALA leading to sleep-promoting neuropeptide secretion is not fully understood . Our forward genetic screens discovered SIS defective mutants with loss-of-function alleles in *ttx-7* gene, the sole inositol monophosphatase homolog. Preliminary data showed that the sleep defects in *ttx-7* mutants are non-developmental since supplementation of *ttx-7's* enzymatic product, myoinositol, fully recovered sleep in adult animals which suggests a signaling defect in the *ttx-7* mutants. In addition, we found that *ttx-7* expression is required in the nervous system, and necessary in the ALA neuron for promoting SIS. We are currently characterizing candidate genes that interact with *ttx-7* to regulate neuropeptide release from the ALA neuron, which will eventually help understanding the role of *ttx-7* in promoting sleep during cellular stress.

902B **The neuroprotective properties of *Lacticaseibacillus rhamnosus* HA-114 in *C. elegans* polyglutamine models of Huntington's disease** Samuel Boyer[1,2], Audrey Labarre[1,2], Alex Parker[1,21]Université de Montréal, [2]crCHUM

Intro: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by a ≥40 CAG repeat expansion in the first exon of the *huntingtin* gene. Despite being monogenic, HD has no disease altering treatments and there is a pressing need to better understand its underlying pathological mechanisms. As the gut-brain axis has garnered increasing interest in research, dysbiosis has been suggested as an important factor in neurodegenerative diseases. Promisingly, it has been demonstrated that the probiotic *Lacticaseibacillus rhamnosus* HA-114 is neuroprotective in HD C. *elegans* models. Yet, the molecular mechanisms underlying HA-114 neuroprotection in HD remains elusive, though mitochondrial dysfunction is thought to play a role. **Objective:** This study aims to further investigate the underlying mechanisms of HA-114 neuroprotection in HD. **Hypothesis:** We propose that the *kat-1*, *acdh-1* and *elo-6* genes implicated in mitochondrial beta-oxidation are necessary for HA-114 neuroprotection in polyglutamine (polyQ) HD C. *elegans* models. **Method:** 40Q and 67Q, transgenic C. *elegans* models expressing pan-neuronal polyglutamine repeats, will be crossed with *kat-1*, *acdh-1,* and *elo-6* knock-out strains to create mutant polyQ strains. PolyQ models show a range of phenotypes including motility problems and neurodegeneration. Thus, in order to identify the molecular pathways necessary for HA-114 modulated neuroprotection, we will test the probiotic for its ability to rescue (suppress) mutant polyQ phenotypes versus the normal E. Coli OP50 diet via paralysis assays and fluorescent microscopy.

903B **Dithianon causes dopaminergic neurotoxicity in *Caenorhabditis elegans*** Sooji Choi, Yuri Lee Hallym University

Dithianon is a quinone-based fungicide that has been widely used in crop management to control a variety of fungal diseases. It may have unintended consequences in non-target species, including humans, due to its multi-site thiol-reactivity. However, research on this topic, particularly on neurotoxicity, has been limited. Here, we investigated the neurotoxic effects of Dithianon and the underlying cellular and molecular toxicity processes using C. elegans as a model organism. Dopaminergic neurons were found to be more vulnerable to chronic dithianon exposure than motor neurons. Dithianon causes dopaminergic neurotoxicity. It also has a negative impact on motility and dopamine-mediated behavior, such as the basal slowing response. Dithianon increases the production of reactive oxygen species as well as mitochondrial fragmentation. It also upregulates the expression of the oxidative stress response gene, SKN/Nrf-mediated *gst-4* and mitochondrial fission-related gene, *drp-1*. Importantly, antioxidant treatment or *drp-1* depletion mitigated neurotoxicity, caused by Dithianon. These findings suggest that Dithianon causes dopaminergic neurotoxicity in *C. elegans* by inducing oxidative stress and mitochondrial dysfunction. It is similar to the degenerative process associated with the pathology of Parkinson›s disease. As a result of our findings, Dithianon is now thought to be a neurotoxicant, with the potential to contribute to the etiology of Parkinson›s disease.

904C **Genetic and Circuit Regulation of Experience-dependent Isothermal Tracking Behavior** Tzu-Ting Huang[1,2], Shunji Nakano[1,3], Hironori J. Matsuyama[1], Yuki Tsukada[1,4], Ikue Mori[11]Neuroscience Institute, Nagoya University, Nagoya, Japan, [2]Graduate School of Life Sciences, Tohoku University, Sendai, Japan, [3]Graduate School of Science, Nagoya University, Nagoya, Japan, [4]Department of Biosciences and Informatics, Keio University, Minato, Japan

Choosing and maintaining an optimal behavioral strategy in an ever-changing environment is essential to maximize the fitness of animals. To address genetic and circuit mechanisms underlying such behavioral transitions, we focused on the *C. elegans* isothermal tracking behavior (IT behavior), a persistent forward-running behavior near a temperature subjected to the previous cultivation experience. On a thermal gradient under their physiological temperature range, *C. elegans* can migrate toward the temperature associated with food experiences, move isothermally along it at the precision of less than 0.1°C differences for up to several minutes, and then persist in this isothermal tracking state for over an hour. Although previous works from our lab and others have revealed principal circuit components required for isothermal tracking behaviors, detailed genetic and neural encoding behind the transition and maintenance of the isothermal tracking state remain unexplored. From a candidate screen for mutants defective in isothermal tracking behaviors, we found null mutants for a putative G-protein coupled receptor kinase gene *grk-1* failed to persist forward-running state and displayed fragmented isothermal tracks across a wide temperature range on a temperature gradient. Despite a broad *grk-1* expression observed in both neuronal and non-neuronal tissues, neuronal *grk-*

1 expression is sufficient and required to maintain the isothermal tracking behavior. Expressing *grk-1*simultaneously in the two interneurons AVA and RIM but not one or the other is sufficient to restore the IT defect of a *grk-1* null mutant. Moreover, inhibiting AVA and RIM neuronal activity of the *grk-1* null mutant via a histamine-gated chloride channel specifically during the navigation on a temperature gradient is sufficient to compensate for the loss of *grk-1* in maintaining IT behaviors. These results suggest that GRK-1 acts in the nervous systems to maintain isothermal tracking behavior partly by inhibiting AVA and RIM neural responses during the navigation. Simultaneous inhibition of AVA and RIM interneurons in the wild-type animals did not further promote their IT behaviors, suggesting that GRK-1 may fine-tune parallel circuit dynamics to optimize IT behaviors in the wild-type animals. Our mutant analyses provide insights into how *grk-1* signaling coordinates neural circuits to maintain an optimal behavioral state persisting over long timescales in a changing environment.

905C **Two distinct interfaces are necessary for synaptotagmin's inhibitory role in synaptic vesicle fusion** Samuel E West[1], Bella N Archibald[1], Matthew L Schwartz[1], Erik M Jorgensen[1,21]University of Utah, [2]Howard Hughes Medical Institute

Fusion of synaptic vesicles to the plasma membrane is driven by the winding together of SNARE proteins. The calcium sensor synaptotagmin blocks fusion by interrupting and restraining the coiling of the SNAREs. The molecular mechanism of pausing fusion is not known. Here we test the role of proposed binding interfaces between synaptotagmin's C2B domain and the SNAREs using targeted editing. We find that residues in two distinct regions of the C2B domain, the 'saddle' and 'arginine fingers', interact with specific residues on the SNARE protein SNAP25 and stabilize the paused state. Mutations of charged residues in SNAP25 contributing to salt bridges in these interfaces resulted in a sensitivity to aldicarb in pharmacological assays. This indicated increased synaptic vesicle fusion and a disruption of the fusion block. Wildtype levels of fusion were restored by introducing reciprocal mutations at charged synaptotagmin residues that interact with the mutated SNAP25 residues; by restoring the salt bridges, albeit with reversed polarities, normal fusion levels were observed. Our results suggest that in the primed state, synaptotagmin blocks SNARE winding by restraining SNAP25 through these two specific binding interfaces. The C2B domain of synaptotagmin binds the plasma membrane and the saddle locks SNAP25 at the bottom of the SNARE bundle, blocking further SNARE rotation. The arginine fingers pull SNAP25 away from the rest of the SNARE proteins, preventing zippering and fusion. We propose a structural model for the primed state of the SNAREs and synaptotagmin and a mechanism for how calcium action on the primed state leads to disinhibition of SNARE winding and fusion.

906C **Molecular mechanisms for activity-dependent insulin signaling that governs sensory neuron development** Nicole A Hall, Lauren Bayer-Horowitz, Candace Linton, Chana Liberow, Niels RingstadNYU Langone Health

How animals behave is determined by what they sense in their surrounding environment. For this reason, the mechanisms that govern development of sensory systems can have a profound and lasting impact on animal behavior. The development of sensory systems begins during embryogenesis, when developmental programs generate neurons endowed with the specialized physiology that allows them to function within a sensory circuit. Also, activity-dependent processes modulate gene expression in sensory systems during and after development. Importantly, activity-dependent gene expression permits the developing sensory nervous system to adapt its function to metabolic or physiological cues.

Caenorhabditis elegans is a powerful model for the study of how neural circuits are defined and refined throughout development. *C. elegans* are richly endowed with diverse sensory neurons that are generated by an invariant cell lineage but whose properties change in response to environmental cues and experience. We previously found that development of the BAG sensory neurons, which detect carbon dioxide produced by respiring microbes, requires regulation of an activity-dependent insulin signal by the p38 MAP kinase PMK-3. To better understand how PMK-3 regulates BAG development, we screened for mutations that suppress the effects of *pmk-3* mutation on BAG fate. Whole-genome sequencing of suppressed mutants revealed that four strains carried mutations in *ebax-1*, which encodes a regulator of axon development. We found that an *ebax-1* Stop-In allele generated by CRISPR also suppressed the *pmk-3* phenotype, indicating that EBAX-1 functions in opposition to p38 MAP kinase during BAG neuron development. Because EBAX-1 is required in motor neurons for axon guidance, we hypothesize that its role in BAG neuron development is to form an axon equipped with the machinery that regulates release of insulin signals. We seek to test this hypothesis by determining the effects of *ebax-1* mutation on the development of synapses in sensory circuits and on gene expression in developing BAG neurons. We hope this approach will identify new molecular players that mediate activity-dependent gene expression in the developing nervous system.

907C **Expansion of feeding state complexity is associated with predatory and cannibalistic behaviours in *Pristionchus pacificus*** Guniz Göze Eren[1], Leonard Böger[1,2], Monika Scholz[3], James W. Lightfoot[11]Genetics of Behaviour, Max Planck Institute for the Neurobiology of Behavior – caesar, [2]Neural Information Flow, Max Planck Institute for the Neurobiology of Behavior, [3]Group Neural Information Flow, Max Planck Institute for the Neurobiology of Behavior – caesar

Neuroscientists have long studied the neuromodulatory systems regulating behavioural states which influence how an organ-

ism's sensory information is processed. In *Caenorhabditis elegans* several persistent behavioural states have been observed which include states associated with food acquisition, locomotion, and sleep-like activities. However, while many behavioural states in *C. elegans* are well defined, evolutionary diverse nematode species have acquired distinct behavioural repertoires. As such, how these behaviours are integrated into their neural circuits and how they may facilitate the emergence of novel behavioural states is unknown. In the nematode *Pristionchus pacificus,* striking feeding behaviours are evident which are not observed in *C. elegans*. Specifically, *P. pacificus* are omnivorous feeders and supplement their bacterial diet by also predating on the larvae of other nematodes. These behaviours are dependent on developmentally dimorphic feeding structures with the eurystomatous (Eu) morph forming two teeth and capable of both predation and bacterial feeding while the stenostomatous (St) morph has a single tooth and only feed on bacteria. In order to investigate these feeding abilities and their potential regulation through distinct behavioural states, we first developed an automated P. pacificus feeding tracker based on the 'Pharaglow' C. elegans system. This was subsequently used to identify specific pharyngeal dynamics associated with either bacterial or predatory feeding in freely moving animals. With this, we have identified distinct feeding and locomotive strategies associated with each morph. On bacterial lawns, Eu morphs move more and eat less compared to St animals which may represent a previously described territorial strategy. Alternatively, in the presence of prey, we only detect killing events associated with the Eu morphs while St animals instead increase their roaming. Furthermore, we have identified distinct and persistent bursts of predatory activity in the Eu morph indicating a predatory behavioural state associated with this mouth form. Therefore, we will next investigate neurotransmitters which may regulate the predatory state switching in *P. pacificus.* Specifically, we will explore any influence of serotonin on the predatory state through the use of *Ppa-tph-1* mutants. Thus, our study will identify how evolutionary divergent behaviours are regulated and their importance for increasing behavioural state complexity.

908C **Transgenerational inheritance of exercise routine: impact on aging-related pathways in *C. elegans*** Aina Bellver Sanchis, Marta Ribalta Vilella, Alba Irisarri, Júlia Jarne-Ferrer, Christian Grinan FerreDepartment of Pharmacology, Toxicology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona

Aging has been defined as a gradual functional decline with a progressive physiological integrity loss, bringing the organism to an increased vulnerability to death. Otherwise, this described deterioration is the major risk factor for most current human pathologies, including neurodegenerative diseases, cancer, cardiovascular disorders, and diabetes.Most important hallmarks of aging have been described as a common denominator of the deterioration process in various organisms. Among them, we highlight genomic instability, epigenetic alterations, telomere attrition, loss of proteostasis, deregulated nutrient sensing, cellular senescence, stem cell exhaustion, mitochondrial dysfunction and altered intracellular communication. Interestingly, exercise training reduces chronic inflammation and age-related oxidative processes, increases autophagy, and improves myokine profile and mitochondrial function, among others. Furthermore, modifications produced by exercise have been demonstrated to escape from reprogramming and to be inherited in sedentary offspring that haven't been in contact with the stimulus, proving the effects of exercise are transgenerational epigenetic inherited. In the present study, we focused on specific age-related pathways and their gene and intermediates' altered expression. We emphasized on CREB transcription factor pathway, IGF-1 signaling, and mitochondrial unfolded protein response (UPR^{mt}) to englobe distant molecular pathways but simultaneously involved in the same longevity, lifespan, and healthy aging processes, to obtain a global view at a molecular level of the aging pathway using *Caenorhabditis elegans (C. elegans)*. Transgenic *C. elegans* strain (PD4251) expressing green fluorescent protein (GFP)-MYO-3, localized in body wall muscles and vulval muscle nuclei, were exposed to a 4-day swimming exercise routine in M9 buffer, and muscle integrity was analyzed by quantification of GFP fluorescence. We found changes in the expression levels of (GFP)-MYO-3 in the treated PD4251 strain in comparison with the control group. Besides, using the thrashing assay as a behavioural assay, we compared the defective motility present at 8 days of age between the treated and the control groups. Likewise, we showed increased gene expression of *Chr1-c*, *Hsp6* and *Hsp60* in N2 treated groups in comparison with the control group. Besides, we analyzed the *Daf2* gene expression, and we found it reduced in the treated group compared to the control group. Interestingly, the muscle integrity and the recovery of the number of thrashes were maintained up to F2. To sum up, our findings demonstrate an easily implemented 4-day swim exercise routine in *C. elegans*, showing an improvement in several age-related pathways, suggesting the beneficial effects of exercise for several age-related pathologies.

909C **AFF-1 mediates auto-fusion between neurites to sculpt a novel toroid morphology in the *C. elegans* I5 neuron** Lachlan Lu, Massimo A Hilliard Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland

Fusion between separate plasma membrane compartments is a fundamental process in the shaping of many cells and tissues, however its potential role in neurons has been largely unexplored. Recent studies have revealed that in some species, neurons have the capacity to fuse their plasma membranes to sculpt their morphology during development, as well as to restore a neurite's morphology after injury. How this occurs, and to what extent it is conserved, is still poorly understood. Here, we characterize the *C. elegans* I5 pharyngeal neuron, whose two neurites have been proposed to fuse during development to achieve a unique seamless toroid morphology. The developmental mechanisms responsible for this morphology, and its physiological significance,

are currently unknown. First, by reconstructing the I5 toroid from serial section electron micrographs we show that it has a continuous cytoplasm indicative of auto-fusion. Then, using fluorescence microscopy, we reveal an essential function for the fusogen protein AFF-1 in I5 toroid morphogenesis. Next, we sought to explore the physiological significance of I5 morphology. We demonstrate that laser ablation of I5 impairs pharyngeal pumping and increases the intervals between pumps. Moreover, we find that I5 exhibits rhythmic, oscillatory calcium dynamics in response to serotonin, and that these oscillations require the SER-7 serotonin receptor. These oscillatory dynamics persist in *unc-13* mutants defective for synaptic transmission, and in *unc-31* mutants defective for neuropeptide signalling, indicating that they may be intrinsically generated. Taken together, these data suggest that rhythmic activity of I5 may play a role in timing and coordinating pharyngeal pumping. Our results uncover a novel mechanism of neuron morphogenesis involving the fusogen AFF-1, and reveal a possible role for I5 in regulating feeding behaviours in *C. elegans*.

910C **Study on the piRNA pathway in axon regeneration** Hyeseon Jeong[1], Uihyeon Yu[2], Jordan Brown[3], Heng-Chi Lee[3], Kyung Won Kim[41]Department of Life science, Hallym University, [2]Life science, Hallym university, [3]Department of Molecular Genetics and Cell Biology, University of Chicago, [4]Department of Life Science, Hallym University

PIWI-interacting RNA (piRNA) are small non-coding regulatory RNAs that interact with the Argonaute protein family's PIWI protein. The PIWI-piRNA pathway is essential for transposable element suppression in germ cells. Furthermore, it has been found to inhibit peripheral axon regeneration in *Caenorhabditis elegans* (Kim *et al*., 2018). In our study, we found that inhibiting the PIWI-piRNA pathway increased the expression of *dlk-1*, an essential regulator known to promote axon regeneration. Here, we hypothesized that PIWI-piRNA pathway inhibits axon regeneration in part by targeting *dlk-1*. To identify *dlk-1*-targeting piRNAs, we predicted piRNA targeting sites on *dlk-1* mRNA by using the piRNA targeting rules (piRTarBase; We *et al.,* 2019) as well as analyzing the neuronal 22G-RNA databases generated by Posner *et al*. (Posner *et al*., 2019). We chose five *dlk-1*-targeting piRNA candidates and used microinjection to create transgenic worms expressing each candidate piRNA. We are currently measuring the levels of *dlk-1* expression and the ability of the candidate piRNAs to regenerate axons. Various reagents were developed in this study to reveal the inhibition of axonal regeneration mechanisms by the PIWI-piRNA pathway, and it will provide insights into the molecular mechanisms underlying the PIWI-piRNA pathway›s inhibitory effects in axon regeneration.

911C **The role of guanylyl cyclases of gustatory preferences in *Pristionchus pacificus*** Vivian Vy Le, Ray L HongBiology, California State University, Northridge

Guanylyl cyclases (*gcys*) are known to help detect water-soluble salts and many receptor-type (rGCYs) are expressed in the ASE amphid sensory neuron in *C. elegans*. We found that there are 5 *gcy-22* homologs in the *Pristionchus pacificus* genome with putative CHE-1 binding sites (ASE motifs). However, this expansion of *gcy-22* is accompanied by a reduction in *C. elegans* ASEL and ASER rGCYs suggests that there are major differences in the expression and utilization of rGCYs between the two species. Our study focuses on determining the degree to which the *gcy* gene family and its signaling pathway is conserved across divergent nematode species. To investigate possible subfunctionalization among the *Ppa-gcy-22* paralogs, we mutated *Ppa-gcy-22.1* by CRISPR/Cas9 and recovered 2 viable reduction-of-function alleles with predicted early truncations to the extracellular domains of the receptor. Interestingly, *Ppa-gcy-22.1* (*csu79*) has enhanced responses to all tested salt cues, in contrast to the *Cel-gcy-22* mutants that show broadly weakened attraction to many salts. We also examined the roles of genes with 1-1 homologs that are expressed in the amphid neurons likely to be involved in the specification and function of gustatory neurons: *Ppa-che-1*, *Ppa-daf-11*, and *Ppa-tax-2*. Whereas *Ppa-che-1* and *Ppa-daf-11* mutants resulted in selective reduction of attraction towards specific salts, we found that *Ppa-tax-2* is necessary for detecting all the salts tested. Additionally, we expressed *Ppa-che-1p::optHisCl* in AM5 and AM6 neurons and found that these ASE and ASG neuronal homologs are involved in mediating attraction towards salts such as ammonium bromide and ammonium iodide. Although we have not yet been able to determine the expression pattern of *Ppa-gcy-22.1* by promoter reporter fusions, we found that another *Ppa-gcy-22* paralog, *Ppa-gcy-22.3,* exhibited left-right asymmetrical expression in an amphid neuron that is not homologous to the ASE and ASG. These preliminary findings suggest that while the functions of the neuronal identity homolog *che-1* and signal transduction effector genes *daf-11* and *tax-2* are conserved, the specific expression pattern and functions of the expanded *Ppa-gcy-22* members may be quite divergent.

912C **Mapping neuronal homology by genetically encoded calcium sensors in *P. pacificus*** Marisa Mackie[1], Kathleen T Quach[2], Ray L Hong[11]California State University, Northridge, [2]Salk Institute for Biological Studies

In *C. elegans,* sensory neurons AWC, ASI, and ASJ are activated upon odor removal. In contrast, other sensory neurons such as the ADL and ASH have been found to show activation upon odor presentation. This brings forward questions about the mechanisms behind these responses, and whether they may be specific to particular neurons or odors. We consider that other nematode species which share homologous amphid neurons with *C. elegans*, such as *Pristionchus pacificus*, might nevertheless exhibit similar neural response dynamics to odorants they do not share. However, it is unknown which amphid neurons in *P. pacificus* correspond to specific odorants. The aim of this study is to examine how the sensory amphid neurons respond to odors in real time, and to map insect-associated odorants to their cognate neurons. Specifically, we expressed codon-optimized

calcium-sensitive fluorescence reporter (GCaMP) in the *Ppa-odr-7* expressing amphid neurons that are homologous to the AWC and ADL neurons. Our results demonstrated that several insect pheromone and plant volatile compounds elicited no response in *Ppa-odr-7p::GCaMP*, suggesting that other amphid neurons might be responsible for sensing those odorants. We are expanding our investigation to include other amphid neurons using the *Ppa-che-1* and *Ppa-odr-3* promoters to drive expression of GCaMP in the ASE and AWA cellular homologs, respectively.

913C **Knockout of *DYRK1A* ortholog *mbk-1* in *C. elegans* provides model for studying key Down syndrome related gene** Elysabeth Otte[1], Emma Bratch[1], Morgan Peters[2], Randall J Roper[1], Charles R Goodlett[11]Biology, Indiana University-Purdue University Indianapolis, [2]North Carolina Agricultural and Technical State University

Down syndrome (DS) is the most common genetic cause of cognitive deficits, affecting ~ 1 in 800 live births. Characterization of DS has focused on identifying potential key genes located on human chromosome 21 (Hsa21), including *DYRK1A*, that contribute to cognitive deficits as well as other symptoms associated with DS in a gene dosage-dependent manner. Mammalian models with knocked-down or triplicated *Dyrk1a* or *Dyrk1a* mutations have identified roles for this key gene; however, complete genetic knockout of *Dyrk1a* from conception is embryonic lethal. This makes studying necessary roles for *Dyrk1a* in early embryonic development difficult. The *mbk-1* gene in *C. elegans* has been identified as an ortholog to mammalian *Dyrk1a*, and genetic knockout of *mbk-1* in *C. elegans* is not lethal. We hypothesize that deletion of the *mbk-1* gene will alter olfaction, learning, and motility in *C. elegans*, and will uncover developmental roles of mammalian *Dyrk1a*.

Using motility, mobility, and preference index assays, an *mbk-1* knockout strain of *C. elegans*, EK228, was characterized to identify potential movement or olfactory deficits when compared to the N2 wild-type strain. Classical conditioning assays were used to determine potential learning deficits associated with the model.

Motility assays conducted on the EK228 and N2 *C. elegans* strains identified no deficits in speed of forward movement. However, the EK228 strain generated fewer body bends in the mobility assay, suggesting a potential role of *mbk-1* in range of motion and muscle control. Preference index assays failed to identify a reduction in olfaction in the EK228 line, contradicting previous findings. Lastly, conditioning assays identified a potential learning deficit in the EK228 line when chemoattractant cues were associated with sodium ion presence indicating a potential role of *mbk-1* in the neural connections associated with associative learning.

Overall, these results imply that EK228 may be a useful model for studying the effects of a knockout of *mbk-1* once the neuronal mechanisms mediating associative learning are identified. EK228 did not display any of the previously described olfactory deficits, and motility did not differ from the N2 strain, making typical assays requiring movement and olfaction for the study of *C. elegans* possible in this strain, including locomotion calcium activity. Further understanding of this model could provide insight into the early embryonic roles of *Dyrk1a* in mammalian development.

914C **Optogenetic induction of Amyotrophic Lateral Sclerosis (ALS) pathology in *Caenorhabditis elegans*** Kyung Hwan PARK Hallym University

Pathological TAR DNA-binding Protein 43 (TDP-43) aggregates that are mislocalized in the cytoplasm, nucleus, or neurites of neurons is a hallmark of TDP-43 proteinopathy, which includes Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). To learn more about TDP-43's role in pathogenesis and neurotoxicity, we used an optoDroplet technique to induce TDP-43 oligomerization through light illumination in *Caenorhabditis elegans*. We created an optogenetic TDP-43 (opto-TDP-43) by fusing TDP-43 with Cry2olig, a light-sensitive protein that undergoes liquid-liquid phase separation (LLPS). When opto-TDP-43 was expressed pan-neuronally, no obvious defect was observed in transgenic worms that were not exposed to light. Surprisingly, when the worms were exposed to light, they developed progressive motor defects as well as shorter lifespans. Opto-TDP-43 formed several inclusion bodies in the cytoplasm of cell bodies and neurites, which were persistent. These characteristics are similar to pathological TDP-43 aggregates seen in ALS patients. These findings imply that TDP-43 mislocalization and inclusion can result in neurotoxicity, affecting mobility and longevity. Overall, we describe the pathological characteristics of TDP-43 proteinopathy in *C. elegans* by creating an optogenetic disease model, and we hope that this model will help us better understand the pathological mechanism of TDP-43 proteinopathy.

915C **Mitochondrial calcium uniporter mediates odor learning and memory through neuropeptide release** Hee Kyung Lee[1,2], Saebom Kwon[1], Kyoung-hye Yoon[11]Physiology, Yonsei University Wonju College of Medicine, [2]Yonsei University Wonju College of Medicine

The known roles of mitochondria in neurons are often limited to their role as ATP suppliers and sources of dangerous ROS. However, recent studies have shown that mitochondria are required for additional, subtle but essential aspects of neuronal function,

such as buffering calcium to fine-tune neuronal activity or maintaining firing rate homeostasis in some neurons. In this study, we aimed to investigate whether mitochondrial calcium plays a role in learning and memory by examining mutants that have a defect in the sole route of calcium entry into mitochondria, MCU-1 (mitochondrial channel uniporter). We used both aversive olfactory learning and positive associative learning paradigms and found that *mcu-1* mutants were defective in odor learning. The defect was restricted to odors recognized by the AWC^{on} neuron. MCU-1 in neurons or in AWC neurons alone was sufficient to restore learning, suggesting that MCU-1 is required in sensory neurons. Pharmacological inhibition showed that MCU-1 was required at the time of learning. Interestingly, the transgenic strain expressing MCU in AWC showed long-term retention of odor memory after only a single training session, in both the aversive learning paradigm and positive associative learning paradigms. This is likely due to higher levels of MCU-1 in the transgenic strain than endogenous levels. This suggests that the amount of MCU-1 in the neuron correlates with learning and memory - a lack of MCU inhibits learning, while too much MCU inhibits forgetting. We noted that similar phenotypes had been observed in the literature in mutants of the neuropeptide NLP-1, its receptor, or in mutants of genes involved in neuropeptide release (1,2). This led us to hypothesize that MCU-1 is required for the control of neuropeptide release in the AWC^{on} neuron. Previous studies show that MCU facilitates neuropeptide release by mediating the production of mitochondrial ROS, which activates protein kinase C (PKC) (3). Consistent with this, we found that treatment with the mitochondrial ROS stressor juglone was sufficient to increase NLP-1 release from AWC. Finally, we found that the *mcu-1* mutant is also defective in salt memory, suggesting that MCU-1 is involved in other forms of learning and memory.

Reference:

1. Chalasani et al 2010. PMID: 20364145

2. Arey et al, 2018. PMID: 29656871

3. Jia and Sieburth, 2021. PMID: 33863916

916C **Microbiome modification: a novel therapeutic for neurodevelopmental disorders** Jing Wang[1], Weijun Feng[1,2], Bing Han[1,21]Children's Hospital, Fudan University, [2]Institutes of Biomedical Sciences, Fudan University

Neurodevelopmental disorders (NDDs) are among the most dreadful diseases, severely hindering the brain functions of >3% pediatric population. To date, few pharmacological or genetic therapies of NDDs has been achieved. During recent years, the concept of the gut-brain axis has emerged, emphasizing the great influences on neuronal functions exerted by commensal bacteria colonizing the digestive track. Therefore, we propose a ground-breaking methodology of engineering gut microbiome to directly cure NDDs of the hosts.

The nematode *Caenorhabditis elegans* is an ideal model to systematically search for effective microbial factors, based on the fact that their gut microbiome is simple and fully controlled in laboratories. Moreover, this organism exhibits considerable genetic conservation with humans, thus facilitating construction of disease models. We have revealed that two nematode mutants at the *chd-7* locus significantly alter the pharyngeal pumping rate, a neuronally regulated response, in opposite directions. This gene is homologous to human chromodomain helicase DNA binding proteins CHD7 and CHD8, the causal agents underlying CHARGE syndrome and certain forms of autism, respectively. These *chd-7* mutants are thus considered as representative NDDs models. Combining with a single-gene deletion library of the model bacteria *Escherichia coli*, we conducted a genome-wide screening before identifying 36 out of the 3985 bacterial mutants ameliorating the impaired pumping of both *chd-7* mutants. Therefore, these 36 deleted *E. coli* genes are potential modification targets to suppress NDDs.

We also tested preliminarily the efficacy of the most potent *E. coli* mutant in a mammalian system. When supplemented to the CHD7 mutant mice, this bacterial strain led to an intriguing recovery from their pathological behaviors. Our findings indicate that a tiny alteration on the microbiome is sufficient to fully antagonize disease progression. The strategy of modifying microbiome genetic background is promising in curing NDDs, displaying enormous potential of medical transformation.

917C **Control of synapse formation by novel extracellular interactions** Morgane Mialon, Liubov Patrash, Jean-Louis Bessereau, Berangere Pinan-LucarreMeLis

The diversity and specificity of synapses rely upon core organizing Cell Adhesion Molecules (CAM) that regulate contact initiation, synapse formation, maturation, maintenance and functional plasticity. We recently identified that the ACR-16 acetylcholine receptor, well characterized at neuromuscular junctions, is also present at neuron-to-neuron synapses along the ventral cord. Using a fluorescent reporter of the ACR-16 acetylcholine receptor, we performed a visual screen upon random mutagenesis to identify mutants with altered ACR-16 containing neuron-to-neuron synapses in the ventral nerve cord. One mutant caught

our attention because the ACR-16 acetylcholine receptor was no longer synaptic and appeared diffuse at the neuronal surface. This phenotype was consistent with a mutation in a core synaptic organizer. We identified the mutated gene, which encodes a member of the Immunoglobulin superfamily CAM. This CAM shows a strikingly specific localization at ACR-16 neuro-to-neuron synapses. Moreover, we found that a known *in vitro* binding partner of this CAM is also very specific of ACR-16 neuron-to-neuron synapses. Overall, our data suggest that we identified two novel synaptic molecules that might form a bridge across neurons and control synapse formation and/or maintenance in *C. elegans*. Interestingly, orthologs of this CAM are associated with a wide spectrum of human neurodevelopmental and neuropsychiatric disorders, and might control synaptogenesis in mammals.

918C **Regulator of lipid metabolism NHR-49 mediates pathogen avoidance and precise control of neuronal activity** Saebom Kwon[1], Hee Kyung Lee[1], Jessica G Antonio[2], Kyoung-hye Yoon[11]Physiology, Yonsei University Wonju College of Medicine, [2]Yonsei University Wonju College of Medicine

Precise control of neuronal activity is crucial for proper function of the nervous system. Failure to maintain appropriate levels of neuronal activity may not only lead to behavioral defects, but could also lead to disease. While investigating the role of the ubiquitously expressed PPAR functional ortholog and lipid regulator NHR-49 in the neurons of *C. elegans*, we found that mutants display defective pathogen avoidance to the human pathogen PA14. Genetic epistasis experiments showed that the defect was downstream of the DAF-7/TGFβ-mediated chemosensory detection of PA14. Transgenic rescue experiments showed that avoidance required *nhr-49* in the neurons, and more specifically, in a set of body cavity neurons that mediate immunity through the G_i-coupled neuropeptide Y(NPY) receptor ortholog, NPR-1. The role of NPR-1 in immunity is well known, and *npr-1* loss-of-function mutants are severely defective for pathogen avoidance. It was previously shown that NPR-1 ligands FLP-18 and FLP-21 are upregulated in response to infection, presumably causing the inhibitory GPCR to lower neuronal activity of the body cavity neurons. We found that FLP-18 and FLP-21 upregulation occurred normally, if not better, in *nhr-49* mutants. Taking all this together, we hypothesize that *nhr-49* mutants fail to lower neuronal activity of the body cavity neurons during infection, which leads to the avoidance defect. Interestingly, supplementation with oleic acid restored avoidance to PA14, demonstrating that the lipid regulator role of NHR-49 is important for mediating this behavior. Moreover, we found that mutant *nhr-49* also suppresses the egg laying defect in the gain-of-function mutant of *egl-6*, another G_i-coupled GPCR in the HSN neuron. This suggests that the role of NHR-49 in suppressing neuronal activity may be a general phenomenon that can be observed in many neurons. We are currently conducting calcium imaging experiments to directly test neuronal activity. This may have implications for understanding how metabolic dysfunction of lipids can contribute to various neuronal disorders that exhibit hyper-excitability, such as epilepsy, chronic pain, and various neurodevelopmental or neurodegenerative diseases.

919C **Characterization of IGEG-1, a sleep-specific EGFR ligand** Jesse G Jones, Bryan RobinsonBiology, California State University, Northridge

Epidermal Growth Factor (EGF) signaling is required for many vital processes across bilateria, from epidermal and cardiac homeostasis in mammals to fertility and vulval development in C. elegans. Given its critical roles, abnormal EGF signaling can be highly deleterious, and unregulated EGFR activity is associated with several human cancers. In contrast to vertebrates, C. elegans is thought to have a single ligand/receptor pair, encoded by *lin-3* and *let-23* respectively, and the study of their roles in vulval development has provided many insights into EGFR signaling. In addition, EGFR signaling mediates C. elegans sleep following cellular damage, a phenomenon known as stress-induced sleep (SIS). Interestingly, while SIS requires *let-23*, *lin-3* is largely dispensable for this process, implying that C. elegans may have another, yet-unidentified EGFR ligand. Supporting this hypothesis, our lab has recently identified an EGF-domain-containing gene, *igeg-1*, in a screen for SIS-defective mutants. Overexpression of IGEG-1 promotes sleep in a LET-23-dependent manner, suggesting that it functions as an EGFR ligand. Like Drosophila VEIN and certain human Neuregulins, the EGF domain of IGEG-1 is preceded by an immunoglobulin-like (Ig) domain. Based on data from these “Ig-EGFs” in other animals, we hypothesize that the IGEG-1 Ig domain plays an important role in facilitating proper EGFR signaling. Here, we investigate the function(s) of the Ig domain of IGEG-1, a newly-discovered C. elegans EGFR ligand.

920C **Dissecting the role of oxidative stress in spinal muscular atrophy: insights from the nematode *Caenorhabditis elegans.*** Paloma Pacheco Torres, Maria Dimitriadi University of Hertfordshire

Spinal Muscular Atrophy (SMA) is the most prevalent paediatric cause of lower motor neuron disease and the most common monogenic cause of death in infancy, triggered by the depletion of the ubiquitously expressed Survival Motor Neuron protein (SMN). It still remains unknown the mechanism for the selective vulnerability of alpha-motor neurons in this neuromuscular disorder. A role of oxidative damage in SMA has been proposed with various SMA models depicting an increase in ROS production and mitochondrial dysfunction. Despite this growing body of evidence, a comprehensive overview of how oxidative stress signalling is perturbed in SMA is still missing.

The aim of the project is to delineate whether oxidative stress contributes to SMA pathogenesis with the ultimate goal to iden-

tify the cellular and molecular pathways needed to spearhead further therapeutic avenues for SMA treatment options. Here we utilise the powerful genetic tools of *Caenorhabditis elegans* in a previously established *C. elegans* SMA model and a range of functional assays, pharmacological challenges, and genetic analysis to delineate the mechanism(s) by which oxidative stress perturbations control SMN function.

Exposure of the *C. elegans* SMA model to compounds that induce oxidative stress through increasing intracellular superoxide levels significantly reduced the survival of the animals, indicating an increased sensitivity to oxidative stress reminiscent to mammalian studies. Furthermore, we combine pharmacological and behavioural assays to study the impact that ROS reduction might have in the SMA neuromuscular phenotype. Our ongoing studies focus on genetic interactions of *smn-1* mutant animals with known players of the oxidative stress pathway and on assays that will further define *smn-1* oxidative stress defects.

The ultimate goal of this research is to gain a deeper understanding of the key cellular events that lead to SMA pathology. Understanding the root cause of SMA is critical for developing effective SMA therapies. Altogether, we highlight the strengths of *C. elegans* as an exceptional tool to understand the molecular mechanisms underlying a devastating motor neuron disease.

921C **A small RNA pathway remotely controls the activities of a sensory neuron in *C. elegans*** Hyeonjeong Hwang, YongJin Cheon, Seunghee Oh, Sujin Jo, Taehyun Kim, Kyuhyung Kim DGIST

Small RNAs regulate gene expression and thus modulate animal behavior. However, the roles and mechanisms of small RNAs in animal behaviors still need to be understood. Here, we show that the small RNA pathway regulates the neuropeptide gene expression in an interneuron to mediate neuronal activities of a sensory neuron-type. *C. elegans* ascr#3(asc-ΔC9, C9) pheromone elicits avoidance behavior in wild-type hermaphrodites (Jang et al., 2012). We found that the exoribonuclease *eri-1* is necessary and sufficient for ascr#3 avoidance behavior; *eri-1* mutants and over-expressed animals exhibit decreased and increased ascr#3 avoidance, respectively. *eri-1* acts in the AVH interneurons to promote the mRNA level of the FMRFamide-like gene *flp-26 in AVH, which* is required for the sensitivity of the ADL-pheromone sensing neurons to ascr#3. Because *eri-1* might function as a negative regulator of endogenous miRNA in metazoan (Thomas et al., 2012), we searched *eri-1* target miRNAs that directly regulate *flp-26* mRNA levels in AVH and found *mir-8207* as a potential target of which roles are being investigated. Our study provides a circuit mechanism underlying small RNAs-mediated behavioral plasticity and helps to understand the roles of small RNAs in animal behavior at the circuit level.

922C **Circuit mechanisms underlying gait switching in *C. elegans*** Kyeong Min Moon, Jihye Cho, Jimin Kim, Kyuhyung Kim DGIST

Animals exhibit distinct locomotion modes referred to as gaits and switch gaits depending upon changes in external or internal conditions. However, molecular and neuronal mechanisms underlying gait switching are not fully understood. The nematode *Caenorhabditis elegans* is a good model system in which to study gait switching because animals exhibit well-defined and flexible locomotive behaviors. For example, animals crawl with low frequency and short wavelengths on solid surfaces (Karbowski et al., 2006) and swim with high frequency and long wavelengths in liquid (Korta et al., 2007; Pierce-Shimomura et al., 2008; Vidal-Gadea et al., 2011). Here we investigate the functional circuit and its mechanisms underlying Crawl-to-Swim (C-S) transition. We found that genetic ablation and optogenetic inhibition of the SMB head motor neurons cause defects in C-S transition and swimming, similar to those observed in SMB-defective *lim-4* mutants, suggesting that SMB regulates C-S transition. To identify additional cells and genes that mediate gait transition, we performed unbiased EMS mutagenesis screens and isolated seven mutant alleles, including *lsk57* and *lsk59*, which are defective in C-S transition and swimming. From whole genome sequencing, we found lesions of *lsk57* and *lsk59* alleles in NALCN channel complex subunit *unc-79* and nAChR non α-subunit *unc-29*, respectively. *unc-79* is expressed in and acts in a few head neurons, including AVA, AVE, RMD, SMD, and RID, while *unc-29* functions in body wall muscles to mediate C-S transition. Together, these results demonstrate that the C-S gait transition is regulated by a neuronal circuit consisting of the *unc-79*-expressed inter-motor neuronal hubs and the SMB head motor neurons, which coordinate muscle activities during and after C-S transition.

923C **Identify the function of Bestrophin calcium-activated chloride channels in *C. elegans*** Jimin Kim[1], Batnasan Sunjidmaa[2], Seoyeong Kim[1], Sujin Jo[1], Hyun-Ho Lim[2], Kyuhyung Kim[11]DGIST, [2]KBRI

Bestrophin is a calcium-activated chloride channel of which gene families are identified in many organisms, including humans in which bestrophins are encoded by the four genes; *BEST1-4*. BEST channels are expressed in multiple tissues and mediate diverse functions, including cell volume regulation (Fischmeister & Hartzell, 2005; Milenkovic et al., 2015). For example, *BEST-1* gene is expressed in the retinal pigment epithelium (RPE), and *BEST-1* mutation is associated with a variety of eye diseases, including best vitelliform macular dystrophy (BVMD) (Marquardt et al., 1998; Petrukhin et al., 1998). However, the exact functions of the bestrophins have not been identified. *C. elegans* has 26 genes of bestrophin (*best-1* to *26*), of which expression patterns and functions remain unknown. We first examined expression patterns of *best* genes and found that *best* genes are expressed

in most, but not all, cell types, including intestine and neuronal cells. We have then expressed BEST channels in HEK293T cells and investigated their electrophysiological characteristics of several BEST channels, including BEST-13, BEST-14, and BEST-19, all of which can be activated by intracellular [Ca^{2+}] increase, confirming Ca^{2+}- activated channels. Interestingly, *best-1* and *best-9* are co-expressed in the pharyngeal-intestinal valve (PI valve), which mediates the pharyngeal plunge. We found that *best-9 best-1* double mutants but not *best-1* and *best-9* single mutants exhibit defects in the pharyngeal plunge; *best-9 best-1* double mutants show increased frequency and length of pharyngeal plunge, which are restored by the expression of *best-1* cDNA under the control of *best-1* promoter, suggesting that *best-1* and *best-9* act redundantly to hyperpolarize the PI valve. This study will uncover roles of BEST channels in *C. elegans* and provide insights into the functions of the mammalian BEST channels.

924C **Identify the function of mechanosensitive channel PEZO-1 in *C. elegans* males** Jihye Cho, Kyeong Min Moon, Sujin Jo, Taehyun Kim, Kyuhyung Kim DGIST

Mechanotransduction allows animals to receive and respond to physical stimuli from environments and mediates many biological processes, including touch, hearing, and proprioception. These processes require a range of specific mechanotransducers to convert mechanical stimuli into biological signals. PIEZO mechanosensitive ion channels have been shown to detect mechanical stimuli and are evolutionarily conserved from bacteria to mammals (Coste et al., 2010). While most vertebrates have two *PIEZO* genes, *C. elegans* has a single ortholog, *pezo-1*, encoding 14 isoforms (Bai X et al., 2020). However, the function of PEZO-1 in *C. elegans* has not been fully understood yet. To investigate its role, we first grouped 14 isoforms depending on the mRNA length and observed their expression patterns. We found that in males, the promoter region of *C. elegans*-specific short isoforms is expressed in several tail neurons, including the 6th ray neurons. Nine pairs of male ray neurons appear to have functional specialization and have been implicated to be involved in mating behavior (Zhang et al., 2018). Interestingly, sensory endings of ray 6 neurons, but not other ray neurons, are not exposed to the external environment (Sulston JE et al., 1980), suggesting a distinct role in mechanotransduction. To identify the function of *pezo-1* in male behaviors (Brugman KI et al., 2022), we analyzed each step of male mating behavior and found that *pezo-1* mutant males are defective in contact response, the first step of mating when the males start ventral contact with their tail and maintain contact for scanning the hermaphrodite body (Liu et al., 1995). Compared with wild-type males, *pezo-1* mutant males spend more time succeeding in contact response. Defects in *pezo-1* mutants are restored by the expression of short and long *pezo-1* cDNAs under the control of *pezo-1* short isoform promoter. Moreover, *pezo-1* mutant males show comparable phenotypes with wild-type males in other mating steps, indicating a specific role of *pezo-1* in male mating behavior. Together, these results suggest that PEZO-1 plays a role as mechanotransducers in detecting mechanical touch during the mating behavior of *C. elegans* males.

925C **Establishing function for ST7, a conserved family of polytopic membrane proteins, in the *C. elegans* nervous system.** Hanna Schoen[1], Niko Amin-Wetzel[1], Ekaterina Lashmanova[1], Sean Flynn[2], Stephen Barratt[1], Mario de Bono[11]IST Austria, [2]Cancer Research UK Cambridge Institute

The highly conserved gene F11A10.5 is the *C. elegans* ortholog of human Suppressor of tumorigenicity 7 (ST7) and Suppressor of tumorigenicity 7-like (ST7L). ST7 genes can be found in the genomes of most metazoa but are absent in the genomes of single-celled eukaryotes. Despite the name, the biological function of ST7 proteins is unknown. ST7 are membrane proteins, consisting of three transmembrane (TM) domains and a large, putatively cytoplasmic domain between TM domains 2 and 3. Initially studies of human tumour-derived cell lines suggested that ST7 is a tumour suppressor gene (Zenklusen et al., Nat Genet., 2001) however subsequent studies have cast doubt on this (Dong and Sidransky, Clin Cancer Res., 2002). In mouse and humans, ST7 and ST7L proteins are expressed broadly, including in the nervous system.

A forward genetic screen for mutants defective in *C. elegans* aggregation behavior identified seven alleles of F11A10.5, including multiple nonsense alleles. A battery of behavioral tests indicate that F11A10.5 mutants, whilst otherwise healthy, have the signature behavioral phenotypes of a disrupted oxygen hub-and-spoke circuit, namely: defects in escape from 21% O_2, elevated escape from CO_2 and robust escape from hypoxia. We tagged endogenous F11A10.5 with mNeonGreen using CRISPR-Cas9. Fluorescence microscopy reveals a predominantly neuronal expression pattern, with most neurons showing fluorescence. To gain insights into where F11A10.5 functions we knocked in a construct encoding GFP and an auxin-inducible degron. The knockin animals were wild type for escape from 21% O_2 and showed the expected neuronal expression pattern. Introducing pan-neuronally expressed TIR1 into the F11A10.5::GFP::AID background did not confer any obvious phenotype in the absence of auxin. Adding auxin to this strain recapitulated the F11A10.5 mutant phenotype. I am now expressing TIR1 in individual and sets of neurons to identify where F11A10.5 functions to promote oxygen sensing behaviors.

Collectively, our data shows that F11A10.5 shapes the oxygen sensing neuronal circuit activity to regulate behavior. We have an opportunity to identify the function of this family of proteins in the biology of healthy and diseased individuals.

926C **Transcription factors driving development of pioneer neurons of the *C. elegans* brain** Laura Sabou EMBL

Brain circuits are tightly organized interconnected networks and the operational complexity of their function reflects the complexity of their architecture and assembly during development. Thus, understanding the principles that determine circuit assembly *in vivo* ultimately allow for a better comprehension of circuit function. An important hallmark in the precision of circuit assembly is its initiation by early outgrowing neuronal processes, termed *pioneer neuron axons*. Despite extensive neurodevelopment studies, the molecular identities of brain-circuit pioneers and the molecular underpinning of their development remain elusive across different organisms. The *C. elegans* brain-circuit, "nerve ring", offers a powerful setting to address these questions, due to its stereotyped anatomy, ease of imaging and genetic manipulations in single-cell resolution.

We previously identified the molecular identities of the functional pioneer neurons in the nerve ring and characterized their interactions with other circuit components to initiate assembly (Rapti et al, 2017). We now studythe molecular mechanisms leading pioneer neurons to drive assembly initiation, how the first pioneering neurons grow and navigate, in a largely axon-free environment and how the identity of pioneers influences their morphogenetic potential. Using candidate genetic approaches and live imaging, we identify an array of transcription factors that are important for the development of pioneer neurons *in vivo.* My research directions aim to understand the spatiotemporal function and mechanisms of actions of these factors, to orchestrate the powerful and important interplay between cell identity and morphogenesis during circuit assembly initiation *in vivo*. Our ongoing work reveals that some homeobox transcription factors drive the morphogenetic precision of pioneers but several other transcription factors families are also important. Moreover, pioneer-neuron morphogenetic factors overlap partially with terminal selectors, while embryonically-expressed transcription factors are also implicated in formation of the pioneer-neurons. Interestingly, some but not all implicated transcription factors are enriched in many pioneer neuron types. Thus, this is not a necessary or sufficient condition for driving pioneer axon bundle formation, suggesting that the different pioneer neuron types cooperate to assembly the pioneer axon bundle. We will report on our strategies and progress in these directions.

927C **Molecular targets and bacterial cures of Alzheimer's disease** Ximing Chen, Bing HanInstitutes of Biomedical Sciences, Fudan University

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative disorders that lacks current effective cures. It has long been realized that the AD pathology is closely related to accumulation of amyloid-beta (Aβ) plaques that exhibit neuronal proteotoxicity. Thus, reducing production of Aβ or organismal detrimental responses to this peptide represents the basic method of antagonizing AD. Therefore, aiming at discovery of novel therapeutics of AD, we used transgenic *Caenorhabditis elegans* that express human Aβ in their muscle as a disease model. Also employing a genome-wide library of *Escherichia coli*, we have found that simply deleting each of four bacterial membrane protein genes, namely *uidC*, *nrfG*, *yfiB*, and *ygjV*, is sufficient to greatly postpone the paralysis and premature death of *C. elegans* caused by Aβ toxicity.

Next, in order to understand the mechanisms underlying this bacterial AD alleviation, we performed transcriptomic analyses on the nematode hosts as well as metabolomic analyses on the bacteria. We then designed an algorithm to identify most likely causal factors from these analyses. In brief, we found that a group of functionally connected nematode proteins are downregulated by all these four bacterial mutants, and a list of commonly over-produced bacterial metabolites predicted to be beneficial for the pathological animals, such as N-acetylcysteine. On the *C. elegans* side, the identified protein group is centered by components of the ubiquitin-proteasome system including many F-box proteins and Skp1 homologues. We confirmed that the trypsin-like proteasomal activities are stimulated by these bacterial mutants using biochemical assays. On the *E. coli* side, we selected three highest-ranked metabolites from our list with identifiable molecular structures before supplementing them to the AD worms. Feeding on each of these pure chemicals resulted in significant remarkable phenotypic improvement, with the one designated as CC12 the most prominent. Moreover, we studied the logarithm dose curve of CC12 to reveal a strict straight line below toxic dosage, with the optimal concentration of 2.3mM.

To summarize, from a unique perspective, we identified the enzymes involved in ubiquitination and proteasomal degradation as promising molecular targets to fight AD pathology, and discovered that several compounds are potential AD curing drugs, awaiting preclinical tests and real-life application.

928C **Autophagy and spinal muscular atrophy: lessons from the nematode *Caenorhabditis elegans*** Saman A Rashid, Michael Hodgson, Maria Dimitriadi School of Life and Medical Sciences, University of Hertfordshire

Spinal Muscular Atrophy (SMA) an autosomal recessive neuromuscular disorder characterised by the degeneration of α-motor neurons in the anterior horn of the spinal cord, resulting in progressive muscle loss and ultimately death. SMA is caused by reduced levels of the ubiquitously expressed survival motor neuron (SMN) protein. Autophagy, a pathway associated with a multitude of neurodegenerative disorders, is dysregulated when SMN levels are depleted, suggesting a potential role in SMA pathogenesis. We aim to define the specific autophagy networks dysregulated in SMA. We first conducted genetic screening of known autophagy markers in the established *Caenorhabditis elegans* (*C. elegans*) SMA model to reveal autophagic defects. We

then challenged the model with RNA interference, knocking down known autophagy related genes. Lastly, we pharmacologically challenged the SMA model, exposing the nematode to modulators of autophagy which are known to target varying steps of the pathway. Genetic screening revealed autophagic defects reminiscent to mammalian models. Furthermore, knockdown of autophagy related genes through RNA interference has defined potential SMA genetic modifiers. Lastly, our drug screen has highlighted compounds targeting varying stages of the autophagic pathway as modifiers of SMA defects. Our results suggest that autophagy is dysregulated in the *C. elegans* SMA model reminiscent to humans; modulation of varying steps of the autophagic pathway has resulted in the amelioration or exacerbation of SMA neuromuscular defects, highlighting different stages of autophagy as potential targets for treatment of the disorder. Future work is required to pinpoint the exact steps at which autophagy is implicated in SMA pathogenesis.

929C **MEF2 transcription factors prevent protein aggregation in dopaminergic neurons** Erick Sousa[1], Seda Koyuncu[2], David Vilchez[2], Nuria Flames[11]Instituto Biomedicina Valencia - CSIC, [2]CECAD Research Center, University of Cologne

In most neurodegenerative diseases protein aggregation is a common hallmark preceding cell death. Currently there are not effective treatments for these pathologies, thus, the identification of factors promoting neuron homeostasis and suppressing pathological protein aggregation is key to design new therapeutic strategies. In Parkinson's disease (PD) patients, dopaminergic neurons of the Substantia Nigra accumulate alpha-synuclein aggregates and eventually die. Despite the biomedical impact of PD, the molecular mechanisms underlying selective susceptibility of dopaminergic neurons to cell death is not understood. Through a whole genome transcription factor RNAi screen we identified MEF-2, the unique MEF2 member in C. elegans, as a transcription factor promoting dopaminergic neuron homeostasis. mef-2 mutants display pathological protein aggregation specifically in dopaminergic neurons, but not other monoaminergic neurons. This pathological phenotype is progressive and requires the presence of dopamine. The human genome encodes for four MEF2 members. Notably, MEF2D levels are decreased in PD patients. In cell lines and mouse PD models modulation of MEF2D activity is downstream several protective and pro-degenerative stimuli. Our results suggest MEF2 transcription factors have an evolutionary conserved role promoting dopaminergic neuron homeostasis and identify dopamine itself as a pro-aggregative factor.

930C **Roles for Olig genes in neuronal and glial specification in *C. elegans*** G. Robert Aguilar[1], Oliver Hobert[21]Department of Biological Sciences, Columbia University, Howard Hughes Medical Institute, [2]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

In vertebrates, the Olig family of basic helix-loop-helix (bHLH) transcription factors play crucial roles in neuronal and glial specification. However, the conservation of this function across phylogeny remains unclear. Here we interrogate the expression and function of all four members of the Olig gene family in *C. elegans* – *hlh-16*, *hlh-32*, *hlh-17*, and *hlh-31*. CRISPR-based reporter alleles show that *hlh-16* is expressed broadly in embryonic glia, and *hlh-16* mutants display partial defects in the differentiation of a pair of unidentified glia. Furthermore, while previous studies using promoter fusions have reported *hlh-17* expression in CEPsh glia, we do not detect this expression in our CRISPR-based reporter. Instead, *hlh-17* and *hlh-32* are both expressed in the AUA interneurons and DB2 and VB2 motor neurons. Mutant analyses reveal that *hlh-17, hlh-31* and *hlh-32* act redundantly to promote the proper peptidergic identity of AUA. Our work reveals roles for Olig genes in neuronal and glial specification in *C. elegans* and fills a gap in our understanding of the evolution of this gene family.

931C **Examining the role of extrinsic factors in post-embryonic nervous system maturation of *Caenorhabditis elegans*** Gabrielle Prince[1], Rebecca Cornell[2], Wei Cao[2], Stephanie Nava[3], Wilber Palma[3], Paul Sternberg[3], Roger Pocock[4], HaoSheng Sun[51]Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, [2]Anatomy and Developmental Biology, Monash University, [3]California Institute of Technology, [4]Monash University, [5]University of Alabama at Birmingham

Across the animal kingdom, post-mitotic neurons undergo extensive maturational changes from birth/hatch to adulthood. However, it remains unclear if these developmental changes during post-embryonic neuronal maturation are controlled by genetic timer programs, extrinsic factors, or by an intersection of gene-environmental regulatory programs. For this project, we focused on examining the effects of extrinsic factors on post-embryonic nervous system maturation. Previous research in vertebrate animals has demonstrated that sensory information from the environment, through neuronal activity dependent mechanisms, is important for sculpting mature neuronal circuits. It is unclear whether post-embryonic neuronal maturation in *C. elegans* is similarly affected by neuronal activity, and by which modes of neurotransmission that altered neuronal activity act through to control post-mitotic neuronal maturation. Using tools that allow for precise spatial temporal control of neuronal activity and gene function, we are systematically disrupting neuronal activity in subsets of neurons as well as disrupting essential components of each neurotransmission pathway to identify the critical mode(s) of neurotransmission, the critical/sensitive period, and the required circuits for post-embryonic post-mitotic neuronal maturation. Our preliminary results demonstrated that disrupted GABAergic neuronal activity as well as neuropeptide secretion from hatch to adolescence impair the maturation of locomotor behavior in adult worms. We are expanding our studies to the investigation of additional environmental stimuli, i.e., early life stress, on post-embryonic neuronal maturation. We found that worms exposed to prolonged starvation during the

L1 stage also demonstrated impaired maturation of locomotor behavior in adulthood. These findings suggest that disruption of neurotransmission and neural activity in early post-natal development, as well as environmental change, leads to behavioral maturation defects in adulthood. Furthermore, we are identifying which molecular pathways are engaged by altered neuronal activity/neurotransmission as a result of environmental changes, and whether they impinge on already identified genetic timer pathways. Our findings provide novel mechanistic insights on how environmental factors control neurodevelopmental trajectory.

932C **Human calmodulin mutations cause arrhythmia and affect neuronal function in *C. elegans*** Magnus Frantzen[1], Helene H Jensen[1], Jonas L Wesseltoft[1], Ana-Octavia Busuioc[1], Katrine V Møller[1], Malene Brohus[1], Palle R Duun[2], Mette Nyegaard[2], Michael T Overgaard[1], Anders Olsen[11]Department of Chemistry and Bioscience, Aalborg University, [2]Department of Health Science and Technology, Aalborg University

Calmodulin is a ubiquitous calcium sensor, involved in processes ranging from cell division and apoptosis to muscle contraction and synaptic transmission. Calmodulin is an exceptionally conserved with only three amino acid substitutions between the human and *C. elegans* protein. In humans, mutations in calmodulin cause cardiac arrhythmia. The arrhythmia often presents early in childhood and can lead to cardiac arrest. Until now, most studies into calmodulin mutations have focused on *in vitro* protein and cellular methods. Using CRISPR/Cas9 we introduced human disease mutations into *C. elegans* and demonstrated that arrhythmic effects of the calmodulin mutations could be recapitulated in disruption of two rhythmic behaviors, pharynx pumping and defecation motor program. Because we see patients with neuronal symptoms, we hypothesized that the cardiac mutations also affected neuronal function. We found that the arrythmia mutations affected neuronal function but in different ways. When treating the worms with aldicarb and levamisole to study the signaling at the neuromuscular junction we found that one mutation increased sensitivity while another had a protective effect. These mutations also affected directionality during migration and termination of the defecation motor program, while a third mutation decreased chemosensing. Together our data demonstrate that human calmodulin mutation have neuronal effects, likely through different molecular mechanism. Future studies will further explore how calmodulin mutations may contribute to neurological diseases.

933C **WrmPxlTrcker: An open-source script to investigate the dynamics of attraction and avoidance behaviors of *Caenorhabditis elegans* populations over large timespans.** Francesca Hodge[1], Thomas P Ilett[2], Netta Cohen[21]Biological Sciences, University of Leeds, [2]Computer Science, University of Leeds

Caenorhabditis elegans is a leading model system for the genetic and neural specification of behaviour. To establish the role of a particular gene, neuron, synapse, or molecular pathway, high throughput, and quantitative assays are required. In particular, to study mechanisms of plasticity and adaptation, a range of assays have been developed, including chemotaxis assays. Several powerful tools exist for tracking *C. elegans* movement, some within the context of navigation and chemotaxis. Many of these rely on object tracking giving detailed information on individuals' movement, however this involves few worms and a high frame rate which can limit experimental duration and increases the computational and storage needs. To address longer term behaviours and plasticity, we sought a robust and flexible software tool that can be used to quantify population movements over time. We developed WrmPxlTrcker -- a simple, low-cost, adaptable python tool, in which the user specifies multiple regions of interest and the indices of interest. Unlike object tracking tools, the software utilises pixel values to quantify chemotactic responses of the populations over timespans of hours. To evaluate our new tool, we assessed temporal changes in attraction and avoidance to given chemical cues and compared these responses between wild type and test animals (in which the relevant sensory neurons are genetically ablated). The software is being prepared for open-source release.

934C **The post-developmental roles of the netrin receptor UNC-40/DCC in health and disease** Sapir Sela, Yehuda Salzberg, Meital Oren-Suissa weizmann institute of science

The netrin-1 receptor DCC is mostly studied for its roles during early nervous system development, however it remains expressed in parts of the mammalian brain into adulthood. Specifically, DCC is expressed in the Substantia Nigra of the adult mammalian brain, in Dopaminergic neurons (DA) which are sensitive to degeneration in the early onset of Parkinson's Disease (PD). However, it is unclear whether and how DCC is involved in the disease. Moreover, in recent years GWAS studies have associated polymorphisms in the DCC locus with multiple adult-onset neurodegenerative disorders, yet DCC's roles in the mature nervous system are still not completely understood.

We utilize *C. elegans* to investigate the role of DCC in neurodegeneration. Surprisingly, in a PD paradigm that uses the neurotoxin 6-OHDA to indce DA neuron degeneration, dopaminergic neurons survived better and showed almost no defects in *unc-40* null mutants compared to WT animals. Thus, *unc-40* loss-of-function alleviates DA neuron degeneration. Remarkably, in animals with over-stabilization of UNC-40, either by CRISPR mutating a degron motif in UNC-40 or by deleting *sel-10*, the E3 ligase that degrades UNC-40, DA neurons degenerated spontaneously even in the absence 6-OHDA treatment, suggesting a detrimental role of UNC-40 in these neurons. Using an RNAi forward genetic screen against factors in different cell-death

pathways, we have also found that UNC-40 stabilization induces parthanatos, an alternative cell-death pathway that has been implicated in the pathology of PD. Finally, we show that the degenerative effect of UNC-40 is only observed in hermaphrodites, and not males, thus reflecting the commonly observed sexual dimorphism of many neurodegenerative diseases. Preliminary results suggests that the stabilization of synapses in a mechanism involving UNC-40 takes part in the degeneration, and that the site-of-action of UNC-40 may originate in glial cells, rather than in the DA neurons.

Taken together, our results reveal new roles for UNC-40/DCC in the mature nervous system, and shed light on its involvement in neuronal health.

935C **Roles of *sax-7* in the long-term maintenance of neuronal architecture** Marin Pascal, Lise Rivollet, Claire Bénard Department of Biological Sciences, Université du Québec à Montréal

After its initial establishment during embryogenesis, neuronal architecture is maintained throughout life in the face of the animal's growth, maturation processes, the addition of new neurons, body movements, and aging. Whereas remarkable advances have uncovered mechanisms that drive nervous system assembly, the processes responsible for the lifelong maintenance of nervous system architecture remain poorly understood. One neuronal maintenance factor that has been identified is the protein SAX-7, homologous to the vertebrate L1 protein family of neural adhesion molecules, which is required for maintaining the organization of neuronal ganglia and fascicles after their successful initial embryonic development. Previous work in the lab generated a true null allele that completely removes the 20 kb of the *sax-7* locus (by CRISPR-Cas9), as well as *sax-7S*-isoform-specific alleles. This null *sax-7(qv30)* is more severe than previously described mutant alleles at least in some contexts. The loss of *sax-7S* largely phenocopies the *sax-7* null, consistent with previous rescue results showing that *sax-7S* is the key isoform in neuronal maintenance. This isoform maintains neuronal organization by acting post-developmentally, as temporally controlled larval transgenic *sax-7S* expression profoundly rescued the null mutants neuronal maintenance defects. Interestingly, most of the protein SAX-7 appears to be cleaved on immunoblots, and we showed that these cleaved SAX-7S fragments together, but not individually, can fully support neuronal maintenance in vivo. We are further addressing the role of these cleaved fragments of SAX-7S by testing their function by single-copy transgenes (MosSCI). We are also testing the implication of *sax-7S* in cells neighboring neurons. The information harnessed by studying the conserved protein SAX-7/L1CAM in long-term neuronal maintenance in the worm may help decipher processes that go awry in some neurodegenerative conditions.

936C **An improved *Caenorhabditis elegans* model of Alzheimer's Disease to monitor neuronal signalling activity** Viktoria Bajuszova[1], Netta Cohen[2], Jamie Johnston[21]School of Molecular and Cellular Biology, University of Leeds, [2]University of Leeds

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disease and is characterised by the presence of Aβ plaques and neurofibrillary tangles which mediate memory impairments due to loss of neurons and impairments in neuronal plasticity. One subset of neurons greatly affected by Aβ toxicity are the glutamatergic neurons. Oftentimes transgenic rodent animals are used to study the toxic effects of Aβ causing these animals to develop unpleasant cognitive impairments. Thus, to reduce and replace the rodent animals used, we are taking advantage of the model organism *Caenorhabditis elegans*. We are developing a *C. elegans* neuronal model of Aβ which will allow simultaneous glutamate and calcium imaging in specific populations of glutamatergic neurons. This new Aβ strain uses a posterior intestinal co-injection marker and has no fluorescence in or near the head region.

Glutamatergic transmission is also known to be required for the olfactory response and indeed one of the earliest symptoms of AD in humans is the loss of smell. A defect in the olfactory response can be recapitulated in *C. elegans* expressing human Aβ in their neurons via a defective chemotaxis towards the odorants benzaldehyde and diacetyl. This defect can be ameliorated via overexpression of the heat shock protein HSP-90 in the neurons. Previously it has been shown that the ameliorative effects of neuronal HSP-90 overexpression are likely mediated via glutamatergic signalling. To identify the specific component of glutamatergic signalling involved in the ameliorative effect of neuronal HSP-90 overexpression on the chemotaxis defect of the neuronal Aβ strain, mutants of specific components of glutamatergic signalling were used. Chemotaxis assays towards benzaldehyde and diacetyl were carried out to determine whether alterations in specific components of the glutamatergic signalling would prevent the ameliorative effect of neuronal HSP-90 overexpression on the chemotaxis defect observed in the Aβ strain.

937C **A tale of two SAMs: *nrx-1* and *nlr-1* function in monoamine neurons to modulate foraging activity** Brandon L Bastien, Michael P Hart Department of Genetics, University of Pennsylvania

Structural and functional properties of neural circuits underlie an animal's behavior. Genetic and environmental stressors that alter neural circuits can lead to changes in behavior, such as those seen in individuals with autism spectrum (AS) and schizophrenia (SCZ). Neurexins and other synaptic adhesion molecules (SAMs) facilitate the development and maintenance of neural circuits, and mutations in SAM genes are associated with AS and SCZ. Here, we quantify individual *C. elegans* activity in the

presence or absence of food using the WorMotel to investigate the role of SAMs in neurons and subpopulations of neurons on foraging behavior, which involves the monoamines dopamine, serotonin, and octopamine. We identified that *nrx-1,* the singular ortholog of human *NRXN* genes, is required in neurons for the proper response to food deprivation. We find the conserved and understudied gamma isoform contributes to initiation of the food deprivation response, in part via octopamine signaling in RIC interneurons, while the conserved alpha isoform contributes to maintenance of the food deprivation response. Thus, both isoforms coordinate the robust and sustained increase in activity in response to food deprivation, which is critical for successful foraging. We also identified a role for *nlr-1/CNTNAP*, a neurexin related receptor gene, in neurons in behavioral responses to food and food deprivation. Loss of *nlr-1* in all neurons reduces a worm's activity on food, but has no impact on the increase in activity in response to food deprivation. Loss of *nlr-1* in dopaminergic, serotonergic, or octopaminergic neurons, however, alters responses to food deprivation differently than loss of *nlr-1* in all neurons, indicating distinct roles for *nlr-1* in subpopulations of monoaminergic neurons that coordinate this behavior. In our ongoing work, we are characterizing roles for *nlr-1* in structural (i.e. presynaptic morphology) and functional (i.e. responses to neuromodulators) properties in these monoaminergic neurons. Collectively, we show that multiple SAMs act within the nervous system to coordinate foraging responses, and we demonstrate that specific expression or loss of these genes in different monoamine neurons leads to unexpected alterations in behavior. This work begins to elucidate how broadly expressed neuronal genes can alter specific behaviors or subsets of behaviors through circuit modulatory mechanisms specific to subsets of neurons.

938C **DBL-1 transforming growth factor-beta signaling protects *C. elegans* from hydrogen peroxide produced by the bacterium *Enterococcus faecium*** Frank Servello, Daniel L Shaw, Vivek Kanpa, Anders Lindberg, Javier Apfeld Northeastern University

The Earth's environment contains harmful reactive chemicals like hydrogen peroxide, produced by some bacteria in concentrations that are lethal to small animals. *C. elegans* possess defenses that confer some degree of protection, including enzymes that break down hydrogen peroxide. However, how *C. elegans* regulates those and other defenses to deal with the lethal threat of H_2O_2 remains poorly understood.

DBL-1, a member of the transforming growth factor-beta (TGF-β) family of signaling proteins, plays an important role in the regulation of various developmental and physiological processes. We found that mutations that reduce or eliminate the function of DBL-1 signaling components make *C. elegans* more sensitive to killing by the environmental peroxide tert-butyl hydroperoxide and by hydrogen peroxide produced by the pathogenic bacterium *Enterococcus faecium*. These components include the DBL-1 ligand, the SMA-6 receptor, the SMAD transcription factors SMA-3 and SMA-4, and the schnurri transcription factor SMA-9. Using tissue-specific rescue experiments, we found that *sma-6(+)* function in the hypodermis or in the intestine is sufficient to increase peroxide resistance in *sma-6(-)* mutants, and that overexpression of *sma-3(+)* in the hypodermis can increase peroxide resistance well above wild-type levels.

Currently, we are analyzing published mRNA-seq datasets [1] to identify SMA-4 transcriptional targets, with the ultimate goal of identifying the defense mechanisms enabling DBL-1 signaling to increase *C. elegans* peroxide resistance.

1. Yu Y, Mutlu AS, Liu H, Wang MC. High-throughput screens using photo-highlighting discover BMP signaling in mitochondrial lipid oxidation. Nature communications. 2017;8(1):865.

939C **Development of a cytoplasmic GABA sensor strain in *C. elegans*** Leonardo Genero[1], Elise Cheynet[2], Marie Gendrel[11]IBENS - ENS - CNRS - INSERM - PSL University, [2]MeLis, CNRS UMR 5284, Univ Claude Bernard Lyon 1

Neuronal circuits rely on a physiological combination of excitatory and inhibitory transmission. In mature neurons, the main inhibitory neurotransmitter is GABA. Traditionally, GABAergic neurons have been classified based on the co-expression of three proteins: i) GAD/UNC-25, glutamic acid decarboxylase, which synthesizes GABA from glutamate, ii) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and iii) GAT/SNF-11, a plasma membrane transporter that recaptures extracellular GABA. In *C. elegans*, only 26 out of 302 neurons were considered GABAergic, until improved immunostaining enabled the identification of 15 additional GABA-positive neurons. Although they stain for GABA and contact neurons expressing postsynaptic $GABA_A$ receptors, they do not all co-express GAD/*unc-25*, VGAT/*unc-47*, and GAT/*snf-11*. In particular, three pairs of neurons express none of those. Thus, they are unable to synthesize, uptake or package GABA the way we know it.

We hypothesize that those neurons use unidentified transporters for GABA uptake and vesicular packaging. If our neurons of interest rely on a transporter to uptake GABA or to load it into vesicles, removing this protein should suppress or increase the presence of GABA in these neurons, respectively. Unfortunately, immunostaining is time consuming. To overcome this issue, we decided to develop a cytoplasmic GABA sensor strain in order to directly assess in living worms the presence of GABA or not. First transgenic lines were obtained and we could see neuronal fluorescence, but not robustly. Optimisation of the sensor strain, such as codon optimisation and pan-neuronal promoter test, is under progress. Thanks to this GABA sensor strain, we will be

able to precisely map all the GABAergic neurons, to assess if the GABA transport is affected in KO mutants for the putative GABA transporter and ultimately to use it for genetic screen.

940C ***C. elegans* relies on SEK-1 and JKK-1 for effective hydrogen peroxide avoidance** Alyson Fulton[1], Yuyan Xu[2], Maedeh Seyedolmohadesin[2], Vivek Venkatachalam[2], Javier Apfeld[21]Biology, Northeastern University, [2]Northeastern University

Hydrogen peroxide (H_2O_2) is a lethal chemical produced by many bacteria in the environment where *C. elegans* lives. This compound damages macromolecules essential for life, including nucleic acids, proteins, and lipids. *C. elegans* avoids H_2O_2, but the mechanisms of avoidance are not well understood. We hypothesized that *C. elegans* might use MAPK pathways to sense and avoid environmental H_2O_2 because these pathways are known to be activated by adverse stimuli in many settings. We measured how well *C. elegans* avoids H_2O_2 in chemotaxis assays. We found that H_2O_2 avoidance was lowered by mutations in *jkk-1*, a MAPKK in the JNK pathway involved in movement coordination, and *sek-1*, a MAPKK in the p38 pathway activated by oxidative stress. We are currently identifying functionally relevant targets and regulators of these kinases, using a candidate gene approach. We are also investigating whether *jkk-1* and *sek-1* are involved in the perception of H_2O_2 by sensory neurons, using brain wide calcium imaging, or function in target tissues controlling the effective concentration of H_2O_2 in the animal.

941C **Visualization of neuropeptide release sites using split fluorescent proteins in *C. elegans*** Eva Dunkel, Ichiro Aoki, Alexander GottschalkBuchmann Institute for Molecular Life Sciences and Institute of Biophysical Chemistry, Goethe University, Max-von-Laue-Str. 15, 60438 Frankfurt am Main

It remains elusive whether neuropeptides (NPs) are released uniformly along neurites or more locally and whether multiple species of NPs expressed in the same neuron are released together or differentially. A nanobody against mCherry was previously fused to a native hypodermal protein to monitor release of mCherry fused to a NP precursor from dendrites of PVD nociceptors (Tao *et al*., 2019). However, intracellular NP::mCherry stored in dense-core vesicles and extracellular, exocytosed mCherry cannot be distinguished with this method – making it difficult to visualize fluorescent proteins (FPs) released from one cell on the surface of another that spatially overlaps. To overcome this limitation and specifically visualize exocytosed proteins in proximity of release sites, we developed a method using split FPs.

We expressed NP precursors fused to a tag and a small fragment of split FPs (NP::tag::FP_{11}) in one neuron and tag-binding proteins (tag-BPs) fused to the other large fragment of split FPs (tag-BP::FP_{1-10}) on the extracellular surface of another neuron. We focused on NP release from the AVK neuron that expresses multiple NPs such as FLP-1, NLP-10 and NLP-49 (Nelson *et al.,* 1998;Chew *et al.,* 2018;Oranth *et al.,* 2018) with distinct functions. We aim to examine if the release sites of these NPs are different. To test if our methodology is functional, we expressed the NP precursors fused with a tag and sfCherry$_{11}$ or spGFP$_{11}$ in AVK and the respective tag-BPs fused to sfCherry$_{1-10}$ or spGFP$_{1-10}$ on the extracellular surface of cholinergic neurons. Fluorescence of sfCherry or spGFP was visible in the nerve ring and along the ventral nerve cord, where both AVK and cholinergic neurons extend processes, but only when both NP::tag::FP_{11} and tagBP::FP_{1-10} were expressed. This indicates that tag-BPs extracellularly captured the tag, leading to the assembly of the split FPs, and that the exocytosed proteins were specifically monitored.

Our methodology should be able to indicate release of any NP of interest on the surface of any nearby cells of interest, even if the cells of the NP origin spatially overlap with cells on which the released peptides are captured. We currently compare the distribution of release sites of two NPs expressed in AVK using spGFP and sfCherry in the same animal. Furthermore, we will identify conditions, and screen for mutants, that may affect release of one, but not other NPs, from AVK.

942C **Voltage imaging reveals defective electrical coupling and aberrant timing in pharyngeal and body wall muscle ensembles in innexin mutants** Christin Wirt, Amelie Bergs, Alexander Gottschalk Buchmann Institute for Molecular Life Sciences, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt am Main

Gap junctions (GJs) function as clustered intercellular channels between neighboring cells in muscular organs and between neurons, allowing ions, molecules and electrical impulses to pass channels. Molecular compositions of GJs affect functional properties like open-probability etc., which determine how cellular networks can be electrically compartmentalized. Previous studies showed that GJs in *C. elegans* are essential for the electrical coupling between cells, and mutations of the subunits (innexins) can cause impaired locomotion (Liu, 2013). To contribute to the understanding of GJ properties and how innexins mediate compartmentalized cellular networks in the pharynx, genetically encoded voltage indicator (GEVI) imaging was used. We compared the wild type (wt) to *inx-6* and *inx-7* mutants, which encode innexins at the 'border' of the compartmentalization domain (metacorpus-isthmus). We found that repolarization overall occurs in a spatiotemporally compartmentalized manner (Azimi Hashemi, 2019) and that the average delay of repolarization from anterior to posterior pharynx differed significantly among the mutants. We also investigated how GJs affect coordination in adjacent body wall muscle (BWM) cells. Here, particularly *unc-9* and *inx-16* showed significant differences in the coordination of muscle cell pairs within an ensemble compared to wt animals, also in the coordination of whole muscle ensembles between genotypes.

We currently explore the use of Channelrhodopsin-2 (ChR2) to photostimulate one cell of the BWM ensemble and image the resulting voltage flow to adjacent cells to analyze the properties of the respective GJs, possibly also uncovering rectification. This approach will be used to also study over-expressed GJ combinations, including a vertebrate connexin, which will allow analyzing how ectopic GJs affect intrinsic muscle cell activity. We also explore using the bidirectional optogenetic actuator BiPOLES to control and clamp voltage by means of our optogenetic voltage clamp (OVC; Bergs, 2023) to investigate the voltage dependence of gating of the respective GJ. We also currently explore to what extent marginal cells contribute to electrical activity of the pharyngeal muscles.
Azimi Hashemi. PMID: 31371514
Bergs. doi: 10.1101/2022.06.03.494532
Liu. PMID 24130800

943C **Regulation of Presynaptic Gene Transcripts During Synaptogenesis** Jackson Rogow, Robert H Singer, Sulagna Das, Peri Kurshan Neuroscience, Albert Einstein College of Medicine

Regulating mRNA in space and time is an ancient, conserved operation cells perform to precisely coordinate the flow of genetic information available for translation. In neurons mRNA transcription, trafficking and local translation are implicated in diverse processes ranging from outgrowth to plasticity and repair. Mutations in several RNA binding proteins are associated with neurodevelopmental diseases and axon defects, yet the role of mRNA regulation in synaptogenesis remains unclear. Additionally, these processes have not been investigated *in vivo* during development. In this project we take advantage of the genetic accessibility and transparency of the nematode C. *elegans* to determine the mechanisms of mRNA regulation of presynaptic active zone genes as synaptogenesis occurs in the living animal. Proof of concept preliminary data suggests that live imaging of both transcription and mRNA trafficking, using the MS2 stem loop and MS2 coat protein system to label mRNA with fluorescent proteins, can be performed in neurons of the intact worm. Ongoing work will optimize imaging resolution and pair this system with synaptic markers to probe the relationship between mRNA regulation and synaptogenesis. Investigating these pathways may identify novel regulatory components and lead to a deeper understanding of neurodevelopment.

944C **Investigating the role of functional diversity of postsynaptic mitochondria in modulating glutamate receptor trafficking** Kaz M Knight[1], Rachel Doser[2], Ennis Deihl[2], Frederic Hoerndli[21]Cell & Molecular Biology/Biomedical Sciences, Colorado State University, [2]Colorado State University

The AMPA sub-type of glutamate receptors (AMPARs) are necessary for excitatory synaptic function. Specifically, dynamic AMPAR transport and localization throughout dendrites is critical for synaptic strengthening as well as learning and memory. We are beginning to unravel the effects of neuronal activity as well as subsequent calcium influx and its downstream signaling on synaptic AMPAR localization. However, how sub cellular compartments, such as mitochondria, contribute to localized calcium signaling, and how they affect AMPAR transport and delivery dynamics are still unknown. Preliminary experiments using *in vivo* imaging of calcium and reactive oxygen species (ROS) in single mitochondria in neurons of intact *C. elegans* animals show that neuronal activity drives mitochondrial ROS formation and is dependent on mitochondrial calcium influx. During these experiments, we observed diversity in calcium influx and ROS production in mitochondria, suggesting potential differences in activity based on mitochondrial proximity to synaptic inputs. To characterize these differences, we express the calcium indicator GCaMP6f inside the mitochondrial matrix and tag the outer membrane with tdTomato to quantify mitochondrial shape and calcium influx after varying optogenetic stimulations of excitatory presynaptic inputs. Future experiments will aim to link unique mitochondrial location and function to distinct synaptic changes in AMPAR content. Furthermore, our preliminary experiments also showed that activity-dependent mitochondrial ROS led to a decrease in synaptic AMPAR delivery and exocytosis. To investigate how ROS levels achieve this, we will turn to key calcium signaling proteins with known oxidizable residues, such as calcium calmodulin dependent protein kinase 2 (CaMKII). In these experiments, we will generate CRISPR mutations in CaMKII residues protecting against oxidation. We will then quantify AMPAR transport and exocytosis dynamics in combination with genetic and optogenetic tools manipulating neuronal ROS such as ctl-2 loss of functions and KillerRed. Overall, our *in vivo* analysis suggests mitochondrial ROS play a critical physiological signaling role affecting excitatory synaptic function. In addition, downstream oxidation of calcium signaling components play key roles in the development of distinct compartmentalized signaling in neurons.

945C **pOpsicle: An all-optical reporter system for synaptic vesicle recycling, combining pH-sensitive fluorescent proteins with optogenetic stimulation** Marius Seidenthal BMLS Frankfurt

pH-sensitive fluorescent proteins are widely used to study synaptic vesicle (SV) fusion and recycling. When targeted to the lumen of SVs, fluorescence of these proteins is quenched by the acidic pH. Following SV fusion, they are exposed to extracellular neutral pH, resulting in a fluorescence increase. SV fusion, recycling and acidification can thus be tracked by tagging integral SV proteins with pH-sensitive proteins. Neurotransmission is generally activated by electrical stimulation, which is not feasible in small, intact animals. Previous in vivo approaches depended on distinct (sensory) stimuli, thus limiting the addressable neuron types. To overcome these limitations, we established an all-optical approach to stimulate and visualize SV fusion and recycling. We com-

bined the pH-sensitive fluorescent protein pHluorin (inserted into the SV protein synaptogyrin) and the novel red-shifted channelrhodopsin ChrimsonSA [1] for optical stimulation. This combination of proteins avoids optical crosstalk. We first tested the pOpsicle assay in neurons of primary cell cultures from dissociated embryos, which allowed estimating the fraction of SVs that is released by moderate optogenetic stimulation to correspond to ca. 20 % of the available SNG-1::pHluorin. Next, we tested this combination in cholinergic neurons and the RIM interneuron pair of intact *C. elegans* nematodes and could unveil differences in SV fusion and recycling kinetics between the two neuron classes. Increase and subsequent decline of fluorescence was further affected by mutations of proteins involved in SV fusion and endocytosis, e.g. synaptobrevin, synaptotagmin, synaptojanin, and endophilin. These results establish pOpsicle (**p**H-sensitive **op**togenetic reporter of synaptic ve**sicle** recycling) as a non-invasive, all-optical approach to investigate different steps of the SV cycle [2]. We are now using this assay to screen putative factors of SV fusion and recycling that we previously identified [3], and to characterize their function at the presynapse in more detail.

1. Oda et al. (2018) Nat Commun 9, 3949.

2. Seidenthal et al. (2022) bioRxiv 12.20.521193.

3. Wabnig et al. (2015) PLoS One 10, e0135584.

946C **Computational dissection of locomotion reveals developmental progression in *C. elegans*** Cera Hassinan[1,2], Scott Sterrett[1], Brennan Summy[2], Arnav Khera[2], Jihong Bai[21]University of Washington, [2]Fred Hutch Cancer Center

Locomotion is a crucial behavior for the survival of animals. It is accomplished by the activation of muscles in specific patterns of contraction and relaxation, which are repeated over time. Recent studies have shown that the crawling behavior of adult *C. elegans* can be reduced to four characteristic shapes, known as eigenworms. This discovery has led to the development of computational techniques to analyze the locomotory patterns of *C. elegans*. In this study, we applied dimensionality reduction techniques to analyze the locomotory patterns of worms at different developmental stages. We examined two major forms of locomotion in *C. elegans*, crawling and swimming. Consistent with previous reports on crawling, we found that the swimming behavior can also be reduced to four eigenworms. However, the characteristic shapes of the eigenworms in swimming and crawling are drastically different, indicating that they are not derived from the same gait sequence. Furthermore, we analyzed the locomotory patterns of worms at five different developmental stages: young L1, late L1, L2, L3, and L4. We found that early L1 animals cannot sustain rhythmic movements, despite displaying basic eigenworm features of swimming and crawling. However, after young L1 and before late L1, the locomotory pattern of developing worms become quickly stabilized. These findings show that the early established patterns of juvenile movements are maintained while the locomotor networks continue undergoing substantial remodeling during development. These data suggest that the neural circuits underlying locomotion are refined during development to enable more stable locomotory patterns. Finally, we investigated the role of Wnt pathways in the development of locomotion. We found that disruption of Wnt pathways caused defects in the development of locomotion. However, only two of the four *wnt* genes, *cwn-2* and *egl-20*, had significant impacts. These results suggest that Wnt signals have specificity in controlling the locomotion development in *C. elegans*. Overall, our study provides a comprehensive analysis of the locomotory patterns of *C. elegans* at different developmental stages and sheds light on the role of Wnt signaling in regulating locomotion development.

947C **Polycomb Repressive Complex 1 components MIG-32 and SPAT-3 contribute to neuronal development, locomotion and exploration behaviours.** Jevithen Nehru, Sofia Eiras, Arneet L Saltzman Department of Cell and Systems Biology, University of Toronto

Nervous system development is orchestrated by precise regulatory mechanisms that dictate gene expression. Histone H2A lysine 119 monoubiquitylation, which is typically associated with gene repression, is deposited by the PRC1 complex and is important for proper neuronal development. The Hermaphrodite Specific Neurons (HSNs) are critical for egg laying and migrate from the tail to the vulva during embryogenesis. The PVQ interneurons are located in the tail and extend axons to the nerve ring in parallel tracts that do not intersect. Mutants of the C. elegans core PRC1 factors *mig-32* and *spat-3* have defective HSN migration and axon projection, and PVQ axons that inappropriately cross the midline boundary (Karakuzu et al. 2009 Development; Pierce et al. 2018 PNAS). Whether these defects are enhanced in *mig-32; spat-3* double mutants, and whether these neuronal defects amount to behavioural changes, is unknown.

We compared the severity and penetrance of the neuronal defects in single and double mutants. We find that defects in PVQ axon migration, but not HSN migration, are enhanced in *mig-32; spat-3* double mutant worms. These findings suggest that *mig-32* and *spat-3* may act in a partially separate manner, despite also being involved in the same complex. Given the defects in neuronal migration and axon projection, we inquired whether the PRC1 mutant worms had altered behaviour. The *mig-32* and *spat-3* mutant worms have decreased thrashing frequency and abnormal movements in liquid media. On solid media, *mig-32* and *spat-3* mutants explore a smaller fraction of their environment, travel a shorter total distance and are more stationary

than wild-type worms over the same time frame. In addition, *mig-32* and *spat-3* mutants are partially egg-laying defective with reduced response to pharmacologic perturbation. We are currently utilizing a tissue-specific auxin-inducible degron system to investigate where PRC1 expression is required for proper neuronal development. Our data suggest *mig-32* and *spat-3* may have both common and distinct functions, and that the neuronal defects in these mutants affect animal behaviour.

948C **The investigation of homeotic identity transformation between pharyngeal neurons** Burcu Gulez, Neda Masoudi, Oliver Hobert Biological Sciences, Columbia University

Terminal selectors specify neuronal fates during neuronal differentiation. In mutants of terminal selector genes, the genes responsible for the original fate are lost, and in some select instances alternative fates are de-repressed, resulting in homeotic transformations. Investigating homeotic transformations can provide valuable insights into the molecular and genetic mechanisms involved in cell fate determination and differentiation across phyla.

We have previously shown that POU/LIM homeobox genes *unc86* and *ttx-3* jointly specify NSM neuronal fate (Zhang et al., 2014). Moreover, in *unc86; ttx-3* double mutants, NSM appears to transform into the identity of the M3 neuron based on morphological and molecular features. We hypothesize that *unc-86* and *ttx-3* together not only drive the differentiation of NSM, but also antagonize M3 glutamatergic cell fate observed via repression of M3 terminal selectors. To test our hypothesis, we are expanding our analysis of the derepression of other M3 fate markers in *unc-86* and *ttx-3* mutants. We also examine whether the expression of M3 terminal selectors is de-repressed in *unc-86; ttx-3* mutants. We already determined that NSM in the absence of *unc-86; ttx-3* shows derepression of *ceh-2*, an Empty spiracles/EMX transcription factor that acts as a terminal selector for M3.

Another transcription factor regulating neuronal identity is the bHLH transcription factor *ngn-1*. Using NeuroPAL and assessing cell body position, we found that in *ngn-1* mutant animals, M2 neurons convert to the fate of their sister M3 cells. Since *ngn-1* is only transiently expressed and, therefore, not acting as a terminal selector, we hypothesize that *ngn-1* promotes M2 and inhibits M3 identity by controlling the transcriptional activation of M2 terminal selectors, which may, in turn, repress the expression of M3 terminal selectors. To test this hypothesis, we are currently defining terminal selectors for M2 differentiation, assessing their loss in *ngn1* mutants and whether their loss also leads to a transformation to M3 identity.

By investigating several homeotic transformation paradigms, we hope this work will further our understanding of the specification of neuronal fate in development.

949C **ODR-10 repression in the AWAs of adult males requires the bHLH transcription factor HLH-3** Kimberly Goodwin, Aixa Alfonso University of Illinois at Chicago

In C. elegans males a subset of sex-shared neurons develops sex-specific functions in the L4 to adult stage transition (Hilbert & Kim, 2017; Pereira et al., 2015; Zhang et al., 1997). The emergence of these dimorphic features is influenced by factors determining sex identity and developmental timing (Lawson et al., 2019). The function of the bHLH transcription factor HLH-3 has been previously demonstrated to be essential for the terminal differentiation of sex-specific neurons such as hermaphrodite specific neurons (HSNs) and ventral cord type C neurons (VCs) (Doonan et al., 2008; Perez & Alfonso, 2020). In this report, we present evidence to extend the role of HLH-3 in the terminal differentiation of the sex-shared sensory neuron pair, the AWAs. These cells are known to have sexually dimorphic features emerge in the L4 to adult transition of males (Ryan et al., 2014). Expression of the diacetyl sensing GPCR, *odr-10*, in the AWAs is down-regulated in well-fed, sexually mature adult males, but remains highly up-regulated in adult hermaphrodites and juvenile males (Ryan et al., 2014). To address whether *hlh-3* influences this sexually dimorphic feature, we determined the intensity of *odr-10p::GFP* in *hlh-3(lof)* adult males. GFP intensity of the cell was measured relative to that of a standard intensity bead. Our results show that *hlh-3(lof)* males show significantly higher relative intensity levels compared to that of the wild type adult males, but not significantly different than that of wild type L4 males. This finding implies that *hlh-3(lof)* AWA cells are stalled in their differentiation, or that a lack of differentiation in another cell is causing a non-autonomous down-regulation of *odr-10*. We will present a possible mechanism underlying this observation.

950C **High resolution view of the dynamics of memory formation** Ithai Rabinowitch, Netanel Cohen Medical Neurobiology, Hebrew University

The nervous system has a remarkable ability to adapt to a changing environment, and even overwrite innate behaviors in beneficial ways. At the neuronal level, such plasticity can manifest itself in various layers of the nervous system, including the establishment of new neural pathways that can alter behavior and adapt it to changes in the environment, requiring, in many cases, gene transcription and translation. The mechanisms and components governing this phenomenon are insufficiently understood. The model animal *Caenorhabditis elegans* is capable of multiple forms of learning and can retain a memory for at least 48 hours. In this work we have developed a novel procedure enabling to condition *C. elegans* in a simple and robust manner

generating a change in behavior that can last several hours following only a few minutes of training. This protocol allowed us to explore the dynamics of gene transcription and translation within a few minutes from the beginning of conditioning, revealing waves of transcription and translation starting immediately after conditioning. Blocking those waves using transcription or translation inhibitors significantly damaged the memory when tested an hour after conditioning. Thus, even a 1-hour memory is transcription- and translation-dependent. To understand more about the identity and function of those proteins we extracted and sequenced mRNA at specific time points following the conditioning. This made it possible to observe the dynamics of gene regulation and identify novel genes that are essential for long-term memory formation, retention and dissipation, revealing relatively rapid transcriptional processes that serve to establish adaptive behavioral plasticity.

951C **WRT-6, a glial hedgehog-related protein, controls sensory neuron properties in C. elegans** Elif Magemizoglu[1], Diana Klompstra[2], Thomas Burglin[3], Shai Shaham[11]The Rockefeller University, [2]Healthcare Consultancy Group, [3]University of Basel

Sensory organs are composed of sensory cells surrounded by glia-like cells, an architecture conserved from invertebrates to humans. The amphid sensory organ of the nematode C. elegans consists of 12 sensory neurons that respond to a panoply of environmental stimuli, as well as two ensheathing glial cells, termed AMsh and AMso glia. This glial pair forms a channel through
which eight neurons send ciliated dendritic endings that contact the environment. Amphid sensory neurons can take up lipophilic dyes, such as DiI, as well as water-soluble dyes, such as FITC, when animals are soaked in these compounds. We recently characterized the transcriptome of AMso glia and noticed that multiple hedgehog-related genes are expressed in this cell. We found that one of these genes, wrt-6, which encodes a secreted hedgehog-related protein, is required for proper uptake of lipophilic, but not water-soluble, dyes by amphid neurons. WRT-6 protein localizes to the AMso glial tip and is required in AMso glia to promote neuronal dye uptake. Structure function studies suggest that the cholesterol-addition site of WRT-6 is required for its function, and that its N-terminal domain is dispensable for activity. Preliminary EM studies reveal defects in amphid channel formation, as well as increased electron density within specific sensory neurons. Ongoing studies aim to identify WRT-6 binding partners, as well as downstream targets of this ligand. Our studies uncover a novel glia-neuron interaction that can be used
not only to understand how glial cells control neural properties, but also to decipher roles for hedgehog proteins in glia-neuron communication.

952C **Lesion conditioning enhancement of neuroregeneration requires CREB and is largely DLK-independent across several neurons in *C. elegans*** Noa Grooms, Amber Chow, Neil Patel, Claire Ma, Samuel Chung Bioengineering, Northeastern University

Despite recent progress, the genetic and cellular mechanisms functionally underlying neuronal regeneration remain incompletely explored, especially regarding the roles of distinct regeneration pathways and cell type. To systematically address these issues, our lab established a multi-neuron regeneration model in *C. elegans*.

Dual-leucine zipper kinase, *dlk-1*/DLK, functionally underlies single-cut “conventional” regeneration in multiple species. In “conditioned” regeneration, the central axon regenerates following injury if there is a second, conditioning lesion of the peripheral sensory axon. We assess the role of the cAMP response element binding protein, *crh-1*/CREB, in conditioned regeneration. While cAMP has long been known to drive conditioning and overactivated CREB is sufficient for enhancing regeneration, the functional role of CREB in mammalian conditioning is unclear.

We test conventional and conditioned regeneration in sensory, inter-, and motor neurons. To test conventional regeneration, we cut the axon once. To test conditioned regeneration, we cut the axon and dendrite concomitantly (bipolar neurons) or the axon twice, 24 hours apart (unipolar neurons). We define the conditioning effect as the difference between regenerated length after one cut and after two cuts. We assess regeneration in wild-type, *dlk-1*, *crh-1*, and *dlk-1; crh-1* ($n \geq 20$ animals each).

The loss of DLK reduces conventional regeneration by 40-100% in multiple neurons yet plays a relatively minor role in conditioning. Crucially, the loss of CREB eliminates the conditioning effect in 7 of 8 cases, independently of DLK. We also show that the conditioning effect correlates with *crh-1* expression.

Our study establishes a baseline capacity for specific forms of regeneration in several neurons. We confirm DLK as a broad, primary driver of conventional regeneration. CREB is well known for its role in learning, memory, and development. We demonstrate a novel role of CREB in functionally underlying conditioned regeneration across all tested neuron types. Our future studies will target known molecules in the well-defined CREB pathways and assess regeneration in several other neurons. We will also correlate our regeneration data with CeNGEN data on neuron-specific gene expression in *C. elegans* to identify candidate genes to assay in regeneration.

953C **The functions of CaMKII and PKC in Wnt-dependent neurite pruning** Menghao Lu[1], Kota Mizumoto[21]Cell and developmental biology, University of British Columbia, [2]University of British Columbia

During development, neurons remove excessive neuronal processes, or neurites, via a mechanism called neurite pruning. Previously, we showed that LIN-44/Wnt instructs neurite pruning of the cholinergic motor neuron PDB by recruiting its receptor LIN-17/Frizzled (Fz) to the pruning neurites in a contact-dependent manner (Lu and Mizumoto, 2019 PMID: 31804181). However, the mechanisms by which Wnt and Fz instruct neurite pruning remain unknown.

We performed candidate screening to elucidate the mechanisms of Wnt-dependent neurite pruning and found that two calcium-dependent protein kinases, UNC-43/CaMKII and PKC-2/PKC, are required for PDB neurite pruning. We observed that the double loss-of-function (lof) mutant *unc-43(e408)*; *pkc-2(ok328)* exhibits a severe neurite pruning defect, similar to the *lin-44(n1792)* single mutant. Neither *unc-43(e408)* nor *pkc-2(ok328)* enhances the pruning defect of *lin-44(n1792)* mutant, suggesting that *unc*-43 and *pkc-2* function in the same signaling pathway as *lin-44.* Consistently, *unc-43(n498)* gain-of-function allele suppresses the pruning defect of *lin-44/wnt* mutant. Furthermore, LIN-17::GFP localization at the pruning neurite is unaffected in the *unc-43(e408)* mutant. These results suggest that *unc-43* and *pkc-2* function downstream of LIN-44 and LIN-17 to regulate PDB neurite pruning.

From candidate screening to identify the calcium channels responsible for activating PKC-2 and UNC-43, we found that the temperature-sensitive mutant of *itr-1(sa73),* the sole ortholog of inositol 1,4,5-trisphosphate receptor in *C. elegans* which releases calcium from the endoplasmic reticulum (ER), exhibited the PDB pruning defect, suggesting that the calcium influx from ER activates UNC-43 and PKC-2 to induce neurite pruning. We are currently examining the local calcium dynamics at the pruning neurites using GCaMP7s in wild-type, *itr-1(sa73)* and *lin-44(n1792)* mutants.

Based on our current results, we propose that LIN-44/Wnt instructs neurite pruning by locally activating UNC-43/CaMKII and PKC-2/PKC. Our study may reveal a novel function of the Wnt-calcium pathway in instructing neurite pruning.

954C **Glutamate and *nrx-1* dependent synaptic changes in an aversive sensory neuron are required for *npr-1* aggregation behavior** Mara H. Cowen[1], Kirthi Reddy[2], Sreekanth Chalasani[2], Michael P. Hart[31]Neuroscience, University of Pennsylvania, [2]Salk Institute, [3]Department of Genetics, University of Pennsylvania

The integration of multiple sensory modalities is a key driver of behavior, which is often impaired in neurological conditions. We leveraged the compact nervous system of *C. elegans* to ask how synaptic molecules contribute to the function of a well-characterized sensory integration circuit (RMG) and the regulation of its behavioral output (aggregation). We found that mutations in the single ortholog of the conserved autism gene, neurexin (*nrx-1*), resulted in a decrease in the number of aggregating worms in a "social/aggregating" background (*npr-1*). Expressing *nrx-1* in subsets or single neurons, we localized the function of the alpha isoform of *nrx-1* in aggregation specifically to the glutamatergic sensory neurons ASH and ADL. Loss of the glutamate transporter, *eat-4*, also reduced aggregation behavior and expression of *eat-4* in ASH and ADL restored this behavior. To explore whether the role of *nrx-1* in aggregation was via glutamate signaling, we used Fluorescence Recovery After Photobleaching (FRAP) to detect changes in the dynamics of glutamate exocytosis in *npr-1* and *npr-1; nrx-1* animals. While glutamate release from ASH was increased in *npr-1* animals compared to N2 controls, this was not dependent on *nrx-1*. We analyzed the number of presynaptic puncta and found that *npr-1* worms had an increased number of ASH synaptic puncta, which was dependent on the presence of *nrx-1*. Therefore, *nrx-1* is required for *npr-1* associated changes in pre-synaptic architecture, but not glutamate release dynamics, in ASH sensory neurons. These distinguishable mechanistic changes align with our finding that *nrx-1* and *eat-4* have an additive effect on aggregation behavior. Lastly, we identified that mutants for excitatory (GLR-1) and inhibitory (AVR-15) glutamate receptors displayed aggregation defects. We are characterizing the function of these receptors in aggregation to determine whether *nrx-1* modulates glutamate receptor expression and clustering at the post-synapse, a known role for neurexins in other systems. This work has important implications for translational studies in that it defines clear roles for *nrx-1* and glutamatergic signaling in controlling a complex circuit mechanism for sensory integration and behavior.

955C **A *C. elegans* happy *meal*: Defining the neurocircuitry of avoidance behaviors to longevity promoting bacteria** Nicole L Stuhr[1], Chris D Turner[1], Sean P Curran[21]Molecular & Computational Biology | Gerontology, University of Southern California, [2]Gerontology, University of Southern California

Organisms utilize sophisticated neurocircuitry to aid in the detection of optimal food sources to maintain physiological homeostasis across the lifespan. Due to the variety of nutrients available in the natural environment, *Caenorhabditis elegans* have evolved optimal foraging techniques to distinguish between detrimental and beneficial microbes in order to thrive and survive in their habitats. *Methylobacterium* is a previously established nutritious and lifespan-promoting bacterial genera that drives faster development and longevity, but despite the many healthspan promoting effects, wild-type *C. elegans* will consistently pick

any other available food to eat suggesting other factors beyond health can supersede healthy choices. In an unbiased genetic screen for Microbes Eaten Alter Life (Meal) we isolated *meal-1* and *meal-2* that suppress the precocious development on *Methylobacterium* without influencing developmental timing on the standard OP50 diet. Here we examine the neuronal and molecular underpinnings associated with development and longevity-promoting food choice behaviors. We employed computational approaches to integrate metabolomic and transcriptomic profiling and identified unique signatures that influence *C. elegans* food choice dynamics. A screen of established regulators of olfaction identified AWB and AWC neurons as critical regulators that mediate this novel diet-gene pair for developmental timing. Taken together, our work defines the neurocircuitry of complex behaviors, like healthy food selection, that can impact multiple life history traits.

956C **Investigation of axonal degeneration pathways in *C. elegans* models of amyotrophic lateral sclerosis** Gilles Tossing[1], Mohamad Oukar[2], Alex Parker[31]Neurosciences, The University of Montreal Hospital Research Centre (CRCHUM), [2]Université de Montréal, [3]Neurosciences, Université de Montréal

Amyotrophic lateral sclerosis (ALS) is a late-onset degenerative disorder characterized by a progressive and selective loss of motor neurons in the brain and spinal cord leading eventually to respiratory failure. ALS is a complex disease with more than 40 genes identified to cause familial ALS in patients, affecting a multitude of cellular mechanisms.

One part of ALS pathology targets the axonal structure, which is the basis of the complex, precise, and fast-firing brain network. The axon is a dynamic structure that responds to various stimuli by synaptic structure modification or axonal retraction, branching, degeneration, or regeneration. These mechanisms are regulated by specific signaling pathways that can be genetically and pharmacologically targeted. These pathways have been studied extensively in mechanically injured axons, which has furthered our understanding of the genetics of axonal regeneration. However, there is a lack of information about the implication of these pathways in neurodegenerative diseases such as ALS, which show early-stage axon degeneration.

From here, we decided to make use of all the available knowledge about axon regeneration to investigate how these pathways act in the context of ALS-induced axonal degeneration and aging.

957C **Multiple peptidergic signaling pathways underlying sensitization and dishabituation in *C. elegans*** Alex Yu[1], Catharine Rankin[21]University of British Columbia, [2]Psychology, University of British Columbia

Nonassociative learning, the simplest form of learning, is thought to help animals selectively allocate attention or neural resources to promote survival. Sensitization and dishabituation are two forms of nonassociative plasticity: both sensitization and dishabituation facilitate the likelihood and/or magnitude of responses. The two forms of learning were once thought to be the same facilitatory process exhibited in different behavioural backgrounds, however, mounting evidence suggests that they are instead two dissociable processes. In the present work, we demonstrated that sensitization and dishabituation are mediated by multiple neuropeptidergic signaling pathways. Using our established paradigms, we found that sensitization of response duration and response speed, two components of the nociceptive response, are regulated by different signaling molecules downstream of FLP-20/FRPR-3. We also found that sensitization and dishabituation of response speed are mediated by the same neuropeptides, however, they appeared to be recruited by different upstream signaling in these two forms of learning. We then tested candidate neuropeptide genes to identify molecules involved in these signaling pathways. We found that a variety of neuropeptides specifically mediate different components of response facilitation in different nonassociative learning paradigms. Taken together, these data show that sensitization and dishabituation can be mediated multiple, distinct and shared, underlying molecular mechanisms, and that sensitization may not be a global, organism-wide, phenomenon, rather, sensitization of different aspects of behavior can be differentially regulated by multiple neuromodulatory pathways.

958C **How worms explore 3D space** Thomas P. Ilett[1], Omer Yuval[1], Felix Salfelder[1], Robert I. Holbrook[2], Thomas Ranner[1], David C. Hogg[1], Netta Cohen[21]School of Computing, University of Leeds, [2]University of Leeds

Caenorhabditis elegans lives in granular and complex fluid habitats which it must explore and forage for survival. However, the nature and mechanisms of its explorations are largely unknown in volumetric environments. In studies of planar motion of *C. elegans*, local area search is well described in terms of tumble and run dynamics consisting of undulations (runs) separated by random turning event. In 3D neither the locomotion primitives nor the exploration strategies are known. We built a high-resolution triaxial imaging systems and captured *C. elegans* young adult locomotion as animals move through a volume filled with a homogeneous gel. A computer vision pipeline is used to reconstruct microscopic postures as well as macroscopic trajectories in the volume. We present a large corpus of reconstructed postures and trajectories and identify novel behaviours. These include non-planar undulatory behaviours (formed by non-planar 3D postures) and non-planar manoeuvres, including new reversals and new turning behaviours. We find that *C. elegans* explores its local volume by combining these 3D locomotion gaits and manoeuvres. Finally, we show that this volumetric exploration can be explained with a simple three-state model whose rates exhibit a strong separation of timescales. Analysis of the model further demonstrates that the strategy employed by *C. ele-*

gans appears optimal for the purpose of exploring the largest enclosed volume within the context of local search. These results demonstrate that hierarchies of timescales and non-planarity are essential components of foraging and survival in the worm›s natural environment.

959C **Lipid extracts from *Rothia* are neuroprotective in a *C. elegans* model of tauopathy** Hiva mesbahi[1], Kim Pho[1], Lesley MacNeil[21]Biochemistry and Biomedical Sciences, McMaster University, [2]Biochemistry and Biomedical Sciences, mcMaster University

Alterations in the microbiota have been observed in many human diseases including diseases of neurodegeneration. However, specific microbiome factors that either promote or protect against neurodegeneration are largely unknown. We are examining the effects of human microbiota in tauopathies, a class of age-associated neurodegenerative diseases that are characterized by the accumulation and aggregation of the microtubule-associated protein Tau. Mutations in the gene encoding Tau (MAPT) result in Frontotemporal Lobal Degenerations (FTLDs). Using a *Caenorhabditis elegans* model of this disease, we examined the impact of exposure to specific bacteria present in the human microbiome on neurodegeneration. We identified strains of bacteria that are neuroprotective in this model of tauopathy, and one that promotes neurodegeneration. We observed that *Rothia* species that suppress neurodegeneration also influence fat metabolism in *C. elegans*. We determined that the ability of *Rothia* species to promote neuroprotection in the PLM neurons requires the fatty acid desaturase *fat-3*. *fat-3* mutants lack D6 fatty acids and are depleted in C20 fatty acids. We are currently investigating the mechanism of *fat-3* dependent neuroprotection and investigating how *Rothia*exposure modifies lipid metabolism.

960C **New insights into neuromodulation mechanisms from the neuropeptide connectome of *Caenorhabditis elegans*** Lidia Ripoll-Sánchez[1,2], Jan Watteyne[3], Haosheng Sun[4,5], Robert W. Fernandez[5], Agoston Mihalik[2], Seth R. Taylor[6], Alexis Weinreb[7], Marc Hammarlund[7], David M. Miller III[6], Oliver Hobert[5], Isabel Beets[3], Petra E. Vértes[2], William R. Schafer[11]MRC Laboratory of Molecular Biology, [2]Department of Psychiatry, Cambridge University, [3]Department of Biology, KU Leuven, [4]Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, [5]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, [6]Department of Cell and Developmental Biology, Vanderbilt University, [7]Department of Neurobiology and Department of Genetics, Yale University School of Medicine

Extrasynaptic signaling modulates synaptically-wired circuits to enable neuronal communication. Indeed, neuromodulatory signals are inherently complex, forming a network that has not been fully described for any animal. To this end, we used single-neuron gene expression from the CeNGEN database along with deorphanization data for neuropeptide-activated G-protein coupled receptors (GPCRs) to generate a draft connectome of neuropeptide signaling in *C. elegans* (see Ripoll-Sánchez *et al*. bioRxiv 2022, 10.30.514396). Over half of the neurons of the nervous system participates in a highly interconnected core of very high neuropeptide degree. Several of the most important nodes this core, (PVT, PVQL, PVQR and PVR) are little-studied neurons that may be specialized for peptidergic neuromodulation. Interestingly, some neuropeptide GPCRs show unexpectedly high co-expression with peptide ligands for other receptors, thus potentially enabling signaling cascades for regulating behavioral states. Furthermore, a high number of autocrine connections are found in the network, especially in the ventral nerve cord indicating a possible role in oscillatory movement control.

The neuropeptide connectome differs greatly in structure from the monoamine, gap junction and synaptic connectomes, with a very high connection density and a decentralized topology. Nevertheless, multiple pairs of neurons are connected in two or more of these connectomes, indicating motifs of interaction between them. As well, combinations of neuropeptide connections correlate with synaptic degree, highlighting an intrinsic topological relationship between both circuits. Finally, despite the high reciprocity of the neuropeptide network, unidirectional neuropeptide connections tend to be found in bidirectional synaptic connections, which could reveal mechanistic clues about how neuropeptides activate latent existent synaptic circuits. We expect that the newly mapped neuropeptide connectome of *C. elegans* and its analysis will serve as a prototype for other animals and provide new insight into the structure of neuromodulatory networks in larger brains.

961C ***C. elegans* avoid a flavonoid, epigallocatechin-3-gallate (EGCG).** YongJin Cheon, Hyeonjeong Hwang, Kyuhyung KimDepartment of Brain Sciences, DGIST

Flavonoids are a class of polyphenolic secondary metabolites found in plants and regulate root development and flower pigmentation. In addition, plants utilize flavonoids as a defense system against microorganisms and even nematodes by acting as repellents. Although flavonoids elicit behavioral responses in nematodes, the molecular mechanisms underlying flavonoid signaling are unknown. Here we report that the free-living nematode *Caenorhabditis elegans* exhibits an avoidance behavior to a flavonoid, epigallocatechin-3-gallate (EGCG). We screened whether flavonoids elicit chemotactic behaviors and found that EGCG, but not other flavonoids (Hesperetin, baicalein, robinetinidin chloride, and quercetin), cause acute avoidance in wild-type hermaphrodites and males. Worms avoided ECG (Epicatechin-3-gallate) but not EGC (Epigallocatechin), indicating that *C.*

elegans avoid galloyl moiety of EGCG. We found that the nociceptive ASH neurons detect EGCG and drive avoidance through the GPCR signaling pathway, including GPCR kinase GRK2, G-protein alpha subunits GPA-3 and ODR-3, TRPV channels OCR-2 and OSM-9. Moreover, *C. elegans* wild isolates differ in EGCG avoidance; several wild strains exhibit decreased EGCG avoidance. We are currently investigating genetic variation underlying a phenotypic difference in EGCG avoidance by comparing the genotype of N2 and EGCG avoidance-defective strains. This study will provide a better understanding of how flavonoids directly affect nematode behavior and shed light on the molecular and genetic mechanisms of defensive strategies in plant-nematode interaction.

962C **Dauers rewire their nervous system to update behaviour repertoire** Mona (Danxuan) Wang[1,2], William Li[2], Alanna Love[3], Ben Mulcahy[2], Daniel Witvliet[4], Sebastian Britz[5], Anna Steyer[6], Stephan Preibisch[7], Yannick Schwab[6], Christian Stigloher[5], Mei Zhen[21]University of Toronto, [2]Lunenfeld-Tanenbaum Research Institute, [3]Molecular Genetics, University of Toronto, [4]Coursera, [5]Imaging Core Facility of the Biocenter, Theodor-Boveri-Institute, Julius-Maximilians-University, [6]Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, [7]Janelia Research Campus, Howard Hughes Medical Institute

To optimize for survival, the nervous system needs to respond to both internal cues, such as hormonal changes dictated by an animal's genetic blueprint, and external triggers, such as food availability. For the newly born *Caenorhabditis elegans (C. elegans)* larva, environmental challenges can halt its reproductive cycle and activate the adaptative developmental program, dauer diapause. Activation of dauer diapause is induced by poor environment or by genetic mutations, such as reduce insulin signaling (InR/*daf-2*), which activates this program regardless of environmental conditions. While dauers exhibit drastic morphological and behavioural changes when compared to non-dauer larva, the form and extent of the global brain rewiring events are unknown.

Using volumetric electron microscopy, we analyzed the synaptic wiring of four dauer brains: three genetically wild-type dauers induced by environmental stress, and one dauer 'induced' genetically by reduced insulin-signaling (*daf-2*). Comparing their wiring with that of 8 brains of non-dauers (wild-type), we found profound morphological and wiring changes. Previous work from our lab shows that connections between interneurons, the core decision-making circuits, are effectively hard-wired, establishing at birth and maintained throughout development for worms undergoing the reproductive cycle. However, some of these hard-wired interneuron connections are altered in dauers, supporting the idea that early life experiences induce critical changes in hard-wired circuits. Interestingly, many changes converge onto a specific group of neurons. Live imaging shows morphological changes are stereotypic across individuals and reversible upon dauer recover, suggesting gene-environment interactions activate synaptic remodeling in *C. elegans.* Furthermore, optogenetic experiments stimulating neurons re-wired in dauers result in altered behavioural responses, suggesting dauer-specific behaviours may be a direct consequence of global nervous system re-organization. Taken together, our work aims to understand how the nervous system reconfigures itself following an environmentally-induced developmental decision.

963C **Investigation of the new serotonin-gated sodium channel LGC-50** Leona Carla Cesar[1], Dimitra Panagaki[2], Iris Hardege[3], Yun Zhang[4], Johanna Höög[2], Julia Morud[21]Department of Chemistry & Molecular Biology, Gothenburg University, [2]Gothenburg University, [3]MRC Laboratory of Molecular Biology, [4]Harvard University

Monoamines are signaling molecules that act through metabotropic or ionotropic receptors across animal phyla. In *C. elegans*, metabotropic monoamine receptors have been extensively studied for their role in modulating neuronal activity through G-protein-mediated second messenger pathways. However, recent studies have demonstrated the existence of additional monoamine activated ionotropic receptors, ligand-gated ion channels, that may mediate synaptic monoamine transmission in *C. elegans*. Ligand-gated ion channels have been found to play a crucial role in transducing sensory information into generating output behavior in animals. One such ion channel is the serotonin-gated sodium channel LGC-50, which in *C. elegans* is expressed in the interneuron RIA. Moreover, LGC-50 in RIA has been shown to play an important role during serotonin-dependent pathogen avoidance learning.

Previous reports have identified a 17-amino acid motif in the intracellular M3/4 domain of LGC-50 to be involved in regulating membrane localization of LGC-50. To investigate this, we use immunoelectron microscopy to observe the exact intracellular localization of LGC-50 in wildtype or mutant worms, lacking the 17-amino acid motif. Furthermore, we have assessed potential binding partners to LGC-50 by performing immunoprecipitation studies. We aim to gain a better understanding of the regulated membrane localization of LGC-50 and the involvement of the 17-amino acid motif, this could shed new light on how receptor plasticity is regulated by ligand-gated ion channels in neural processes and by so identifying new molecular mechanisms underlying learning and memory formation.

964C **Multifactorial *Caernohabditis elegans* depression model** Dianelena Eugenio-Pérez, Estefani Yaquelin Hernández-Cruz, José Pedraza-Chaverri Biology, Faculty of Chemistry, UNAM

Current animal models of psychiatric diseases do not adequately model these pathologies mainly because they do not share the complex etiological origin of psychiatric illnesses. In this regard, a *C. elegans* model could be an alternative since combinations of

genetic and environmental factors can be easily modelled. Furthermore, because of its short life cycle (3 days), factors affecting different developmental stages can be included in experimental models.

This poster will describe a proposal for a *C. elegans* depression model that includes both a genetic and an environmental factor, the latter being applied in the first larval stages (L1-L2), as well as in mature adults. The genetic factor is the usage of a knock-out *skn-1c* strain. Notably, the product of the human homologue gene *NRF-2* is downregulated in depressed patients. Concerning the environmental factor, we repeatedly induce the flight response in worms by periodically tapping their Petri dish with a microcontroller-driven solenoid. This repeated induction of stress response models chronic stress, which is a relevant predictor of depression. Importantly, this factor is applied in early life and adulthood because early life stress is known to sensitize adults to further stress that leads to depression.

In this work, we will present detailed results on the impact of each of these factors and their combination in motivated behaviour of *C. elegans* adults, specifically in motivated food seeking, where worms are food-deprived and spontaneous movement for food seeking is assessed.

966C **Investigating epilepsy using a novel *unc-49* ($GABA_A$ receptor) mutant.** Ami Gadhia, Alan Morgan, Jeff Barclay University of Liverpool

Genetic epilepsy accounts for approximately 30-40% of total epilepsy cases; however, often the genes involved, and pathological mechanisms remain elusive. A landmark paper established that missense mutations in human $GABA_A$ receptors are a common cause of genetic epilepsy. In our study, we aim to create a humanised worm model that can be used to study novel mutations. The worm homologue for the human $GABA_A$ receptor is called *unc-49* and is required for coordinated locomotion. Via a singular promoter, *unc-49* generates three distinct subunits (*unc-49*A/B/C) which co-assemble to form a heteromeric receptor. Many *unc-49* mutants have previously been scrutinised; however, none are complete true deletions. Therefore, in the pursuit of creating a null mutant, we utilised the CRISPR-Cas9 technique to produce an *unc-49* knockout. Using a variety of behavioural assays, we confirmed that our novel null mutant elicits the same phenotypic deficits as standard known *unc-49* mutants. We next questioned whether we could rescue the null mutant by introducing wildtype *unc-49* cDNA; whereby, the endogenous *unc-49* promoter was driving the expression of *unc-49B*. Functional analysis showed that a partial rescue effect was achieved, whereby the rescue strain outperformed the null mutant, but did not fully resemble wildtype. Furthermore, incorporation of human $GABA_A$receptor cDNA resulted in similar rescuing abilities. Combined, such results suggest that despite the partial completeness of our rescue strains, we can produce a viable humanised worm model. Finally, *in vivo* we investigated two novel epilepsy-linked mutations, that were produced in endogenous *unc-49* using CRISPR-Cas9. The genetically clean point mutants C167W and G254D, both identified in the human *GABRA1* gene, were selected due to high conservation between the human and *C. elegans* genomes. Functional analysis found that both mutants displayed phenotypic defects that are consistent with those seen in the *unc-49* null mutant, suggesting that they have the capacity to be pathogenic. In conclusion, we have produced and phenotypically assessed our true *unc-49* null mutant which can be partially rescued using wildtype *unc-49* or human $GABA_A$ receptor cDNA. We also found that human epilepsy-linked point mutations displayed phenotypic deficits consistent with what we saw in the *unc-49* null mutant. Such findings highlight the possibility of using *C. elegans* as an efficient way of investigating novel mutations to assess pathogenicity.

967C **Determination of the ADSL deficiency-directed perturbation and mechanism impacting the changes in tyramine level** Sabrina A Sony, Wendy Hanna-Rose Biochemistry and Molecular Biology, The Pennsylvania State University

Adenylosuccinate lyase deficiency (ASLD) is a rare disorder that causes neurological and muscle dysfunction, behavioral abnormalities, and autistic-like symptoms. Our lab has been using *Caenorhabditis elegans* to investigate the neurodevelopmental function of the *adsl-1* gene and found that disruption of the gene using RNAi results in a novel, altered learning phenotype. In addition, *adsl-1(RNAi)* also leads to a change in metabolite levels in tyrosine metabolism, and supplementing *adsl-1(RNAi)* animals with tyramine reversed their learning phenotype. The molecular mechanism that links perturbations of purine biosynthesis and tyramine reduction is still unknown. My central hypothesis is that the loss of ADSL function leads to substrate SAICAR accumulation and purine depletion. As a result of this metabolic change(s), the reduction of tyrosine decarboxylase (TDC) enzyme is responsible for less tyramine. To test the hypothesis, I have used genetic and pharmacological approaches to manipulate SAICAR levels in *C. elegans* and compare their associative learning behavior using a gustatory plasticity assay and locomotive behaviors by head oscillation assay. EV(empty vector) and *adsl-1(RNAi)* animals were supplemented with LMX (lometrexol) in the first stage (L1) larvae. In the presence of LMX*, adsl-1(RNAi)* animals behave normally in both assays, which supports that reducing SAICAR can rescue the phenotype. The results indicate that *adsl-1(RNAi)'s* aberrant behavior can be attributed to SAICAR toxicity. Measuring SAICAR levels using LC-MS is to be completed. Then I can conclude if SAICAR is one of the primary metabolites imposing the altered learning & locomotive phenotype. The methodology also involves using qPCR to measure the *tdc-1* gene expression level and TDC enzyme activity assay to identify the presence and quantity of the enzyme.

LC-MS will be used to measure purine and tyramine levels to correlate them with the phenotypes. This study aims to determine (i) significant metabolic change(s) that correspond with tyramine depletion and (ii) possible mechanisms leading to loss of TDC enzyme activity. These findings will fill the gap in knowledge about how the lack of ADSL enzyme leads to changes in phenotypic outcomes.

968C **Brain-wide representations of behavior spanning multiple timescales and states in *C. elegans*** Adam Atanas[1], Jung-soo Kim[2], Ziyu Wang[2], Eric Bueno[2], McCoy Becker[2], Di Kang[2], Jungyeon Park[2], Cassi Estrem[2], Talya Kramer[2], Saba Baskoylu[2], Vikash Mansinghka[2], Steve Flavell[31]Computational and Systems Biology, MIT, [2]Department of Brain and Cognitive Sciences, MIT, [3]MIT

Changes in an animal's behavior and internal state are accompanied by widespread changes in activity across its brain. However, how neurons across the brain encode behavior and how this is impacted by state is poorly understood. We recorded brain-wide activity and the diverse motor programs of freely-moving *C. elegans* and built probabilistic models that explain how each neuron encodes quantitative features of the animal's behavior. By determining the identities of the recorded neurons, we created, for the first time, an atlas of how the defined neuron classes in the *C. elegans* connectome encode behavior. Many neuron classes have conjunctive representations of multiple behaviors. Moreover, while many neurons encode current motor actions, others encode recent actions. Changes in behavioral state are accompanied by widespread changes in how neurons encode behavior, and we identify these flexible nodes in the connectome. Our results provide a global map of how the cell types across an animal's brain encode its behavior.

969C **Lateralization of axonal growth in the embryonic nervous system** Khulganaa Buyannemekh[1], Paul Villoutreix[2], Vincent Bertrand[11]Institut de Biologie du Development de Marseille, CNRS, Aix-Marseille University, [2]Laboratoire d'Informatique et Systemes, CNRS, Aix-Marseille University

Although the nervous system is mainly left-right (L-R) symmetric at the structural level, some molecular and functional L-R asymmetries exist. Defects in such asymmetries can lead to neurological disorders such as dyslexia in humans. However, the extent of these molecular L-R asymmetries and their functional consequences remain poorly characterized. *C. elegans* is a good system to study this process as its nervous system is simple, and the position and lineage of every neuron have been mapped. We have previously identified a bHLH transcription factor, HLH-16, that is L-R asymmetrically expressed in a small subset of morphologically symmetric interneurons and motor neurons in *C. elegans* (AIY, SMDD, SIAD and SIBV). Interestingly, this asymmetry in gene expression results in L-R functional differences in axonal growth. However, the downstream targets of HLH-16 responsible for this L-R asymmetric phenotype remain to be determined. Here we identify a member of the ephrin family, EFN-2, as a downstream target. We show that this ephrin is L-R asymmetrically expressed and regulates axonal growth in a L-R asymmetric manner along with other ephrins. In addition, using single-cell RNAseq data analysis, we identify another candidate with L-R asymmetric expression, the Flamingo protein FMI-1. Currently we are investigating the underlying mechanism of how ephrins regulate lateralization of axonal growth and are characterizing the role of Flamingo in this process. By identifying novel molecular L-R asymmetries in the nervous system and their functional consequences, our study could help in better understanding brain asymmetries in general, as well as neurological disorders associated with defects in such asymmetries.

970C **Behavior transition in no-reward situations** Shiori Onoue[1,2], Koji Kyoda[2], Shuichi Onami[1,21]Graduate School of Frontier Biosciences, Osaka University, [2]Center for Biosystems Dynamics Research, RIKEN

C. elegans have a lot of favorite odorants. Most of these attractive odorants are natural products of bacterial metabolism, the worm feed. We hypothesize that in the chemotaxis behavior, worms expect the presence of feed where the attractive odorant is and toward. The behavioral mechanisms of how to move toward attractive odorants are well known. It remains unknown in detail how to behave if there is no feed where worms expect the presence of feed by attractive odor. We try to elucidate the unknown mechanisms of how worms behave in unexpected no-feed situations. To quantitatively analyze worm behavior, we developed a new worm tracking system, "Simple Worm Tracker (SWT)," which quantitates worm trajectories automatically from movies. We applied SWT to movies of chemotaxis behavior for attractive odor isoamyl alcohol (IAA); a 6 cm plate, every 0.04 s for 50 minutes, was taken using a digital 4K single-lens reflex camera. Then we measured these trajectory data on how far away from IAA the worms were at each time point. Our results suggest they have two behaviors in chemotaxis; one is approaching, and the other is leaving from IAA. Besides, the worm›s moving speed leaving from IAA is faster than free moving. We plan to analyze worm trajectory in more detail to investigate what mechanisms underlie worm behavior transit of approaching to leaving an attractive odor, from the viewpoint they faced in expect and no-reward.

971C **Loss of the OSM-9 TRP Channel Protein Disrupts Sleep-Dependent Olfactory Memory** Kevin C Daigle[1], Mashel Fatema A Saifuddin[1], Julia M Miller[1], Kelli L Benedetti[1], Rashmi Chandra[1], Alec Chen[2], Christine Lin[2], Angel Garcia[1], Burhanuddin Calcuttawala[1], Angelica Tovar[1], Jackson Borchardt[1], Raymond L Dunn[1], Julia A Kaye[1], Saul Kato[1], Bo Zhang[3], Maria E Gallegos[4], Torsten Wittmann[1], Noelle L'Etoile[11]UCSF, [2]Lowell High School, [3]Washington University, [4]Cal State East Bay

Major molecular processes underlying memory formation are still unknown. By understanding how the key molecular players required for learning and memory function during these processes, we hope to reveal what molecular oddities might limit the neuroplasticity required for memory formation and consolidation. Currently, we know that short term memory is stored in AWC via transcriptional changes (Juang *et al.*, 2013) and possibly downstream in changes in signaling between AWC and AIB (Cho *et al.*, 2016) while long lasting memory may reflect changes in the connection between AWC and other downstream interneurons including AIY (Chandra *et al*., 2023). We found that loss of function mutations in the TRP channel OSM-9 blocked learning and long term (16 hour) memory in *C. elegans*. Though TRP channels are known to be integral parts of chemical, thermal and mechanical perception, how they might affect stimulus integration is not clear. To address this question we not only examined mutants that lack the channel but we also asked where the channel is normally expressed and what happens to butanone sensation in animals that lack the channel. Here we show that predicted loss-of-function OSM-9 mutants fail to learn to avoid butanone and fail to keep the weak memory they have. The osm-9 alleles ky10, yz6, n2743 perturb learning (memory at t=0) and long term (16 hour) memory. n1516 and ok1677 learn and consolidate like wildtypes. This reveals three things: **1.** The loss of osm-9 (null alleles ky10 and yz6) perturbs learning and memory. **2.** Neither the ankyrin repeats nor the deletion of the 2nd and 3rd to last exons are important for osm-9 function in long term memory. **3.** However, the third ankyrin repeat is important for animals to avoid butanone after 3 cycles of training. Imaging shows that endogenously (CRISPR) tagged OSM-9 is expressed in ~20 neurons throughout the animal and levels of OSM-9 do not change with training. We also discovered that OD2772, which is the loss of OSM-9 in ADL and ADF, results in loss of learning and memory to avoid butanone. Preliminary studies examining brain-wide calcium activity in naive ky10 animals show that more cells are responsive to butanone than in N2. This indicates that loss of OSM-9 can lead to more butanone responsive activity in cells in addition to the ones that are responsive in N2. These cells could be OSM-9 expressing cells and/or others that compensate for the loss of OSM-9. Thus, osm-9(lof) could be a neomorphic mutant that paradoxically promotes butanone sensation in cells that do not normally respond to that odor. These cells may not be able to adapt to the odor, interfering with odor learning and memory. We will be exploring this possibility. This could indicate that the animal compensates for the loss of OSM-9 by up-regulating responses to butanone. This provides insight that loss of OSM-9 is a molecular oddity that limits the neuroplasticity required for memory formation and consolidation.

972C **The progeny of C.elegans that have been exposed to high salt conditions in its parent generation avoid high salt conditions** Manami DoteUniversity of Tokyo

Recent study reveals that learning of avoidance behavior toward pathogenic bacteria in C. elegans is inherited. Other than the avoidance of pathogenic bacteria, the effectiveness of food exploration is important for survival. C. elegans is attracted to odor in order to efficiently probe for food with essential nutrients. It is known that siRNA must be synthesized to find food efficiently. Non-coding RNAs including si RNA play an important role in epigenetic controls. C. elegans might pass the information about the feeding environment in which they grew up to their progenies to affect the behavior of the progenies through non-coding RNA.

C. elegans is attracted to salt(NaCl) after exposure to the salt with food. We discovered that, after C. elegans have been exposed to high salt conditions (100mM NaCl), their progenies avoid high salt conditions. Also, exposure to higher salt conditions (200mM), we observed further avoidance of high salt conditions. The progeny whose parents were exposed to low salt condition behave normally, suggesting the inheritance of behavior only occurs when C. elegans is exposed to strong salt stimulus. Two generations of exposure to high salt conditions did not show further avoidance. To eliminate the possibility of osmotic stress effects, we used sucrose, which has little effect on salt chemotaxis, to keep the same osmotic levels. However, C. elegans did not show abnormalities in chemotaxis. Thus, we assume that the information of the environment that parents lived in is passed down to their progeny. We also found exposure to high salt conditions in L1 is required for the inheritance of the behavior. Moreover, the environment in which the adult was placed does not affect its inheritance. L1 imprinting might be one of the factors for the inheritance.

In C. elegans, heritable small RNA is transmitted through generations to regulate gene expression. Moreover, small RNA synthesis is required for effective food exploration in C. elegans. C. elegans may store information about their feeding environment in the small RNA. It is possible that they are attracted to an environment different from the one in which their parents grew up in order to find a new feeding ground.

973C **High throughput functional metagenomic screening in *C. elegans*** Bonnie Evans[1,2], Andre Brown[1,21]Behavioural Phenomics, MRC London Institute of Medical Sciences, [2]Institute of Clinical Sciences, Faculty of Medicine, Imperial College London

Soil bacteria are a potentially rich source of novel compounds with agricultural or therapeutic use. However, many strains require specialised or as-yet undetermined growth conditions. Metagenomic libraries are constructed by cloning DNA fragments isolated directly from soil microbial communities into easily culturable hosts, thus allowing the biosynthetic potential of unculturable strains to be accessed.

We screen metagenomic clones for effects on *C. elegans* using a high throughput imaging system. A set of behavioural features, including worm posture and speed, are extracted from recordings to quantitatively phenotype *C. elegans.* Behavioural phenotyping can be used to reveal subtle differences in the effect of metagenomic clones, which can be directly linked to the encoded product of metagenomic DNA.

974C **The role of Whole Brain Calcium Dynamics During Memory Consolidation** Mashel Fatema Saifuddin[1], Kevin Daigle[2], Julia Miller[3], Raymond Dunn[2], Fernando Munoz-Lobato[2], Kelli Benedetti[4], Rashmi Chandra[2], Itamar Lev[5], Sarah Nordquist[21]TET-RAD, UCSF, [2]UCSF, [3]UCLA, [4]Genentech, [5]University of Vienna

Memory provides an important survival benefit across many species, as failure to recognize signals associated with harmful agents can be deadly. We know that *C. elegans* can learn to associate an inherently attractive concentration of butanone with starvation (Kauffmann 2011). We have shown that this associative memory can last up to 24 hours if it is immediately followed by two-hours of sleep (Chandra et al., 2023). Though sleep after learning allows animals ranging from humans to nematodes to permanently store memory, it remains unclear how neural dynamics during this period allows memory consolidation.

In order to understand what neural dynamics are important for memory consolidation, we are examining the butanone-responsive behavior of animals during this period at the level of a. chemotaxis and b. neural dynamics of the entire anterior nervous system. We will present our findings that: a. the association between butanone and starvation is lost if the animals are tested after being on food for < 90' but if they are allowed to feed for >120' they retain the memory. b. We will present the calcium activity patterns evoked by butanone presentation of an animal that is attracted to butanone (buffer-trained control). We will compare that with worms that are indifferent to that odor (butanone trained). We expect that the animals that seek out butanone will show the neural correlates of reduced turns and backing during butanone exposure. By contrast, if the animals have learned to avoid butanone, those animals will increase turns and backing and their neural correlates would reflect that. The neurons are differentially active upstream of these motor plans are proposed to identify neurons important to the butanone-indifferent response of an animal that has consolidated memory. This unbiased approach will identify neurons that we can next prove are important for consolidation by conditionally ablating their activity and asking ho what affects the neural dynamics and behavior of the animals. Finally, we will ask what calcium activity patterns are present in a sleeping animal versus an animal who has been disturbed using butanone presentation during the labile period. This may reveal new patterns of activity that could explain how loss of sleep can block consolidation. Examining the patterns of activity within the butanone-sensing circuit as well as the interactions with other neuronal ensembles will provide us with important insight into the how decision-making process are consolidated during sleep after learning.

975C **Serum- and Glucocorticoid- inducible Kinase 1 (SGK-1) is a master regulator of neuropeptide secretion in *C. elegans*** Sebastian Ciscares Velazquez[1], Martin Piringer[1], Gang Wu[2], Yijian Yan[2], Ralf Baumeister[2,3], Stefan Eimer[11]Goethe University Frankfurt, [2]Bio3/BioInformatics and Molecular Genetics (Biology), Albert-Ludwigs-University Freiburg, [3]ZBMZ/ Center for Molecular Medicine and Cell Research (Medicine), Albert Ludwigs-University Freiburg

More than 250 neuropeptides (NPs) have been identified in *C. elegans* and have been shown to modulate neuronal activity and behavior. However, little is known about the basic intracellular mechanisms regulating NP release from dense-core vesicles (DCVs) and their metabolic modulation. The serum- and glucocorticoid-inducible kinase 1 (SGK-1) is expressed in the intestine and modulates stress responses and lifespan. We describe here a neuronal activity of sgk-1 that might link the perception of environmental stimuli to their metabolic responses in the intestine. Using *in vivo* assays, we found that SGK-1 function is essential for the secretion of NP from DCVs. Whereas *sgk-1(-)* prevents NP secretion, a constitutively active mutant form of SGK-1 expressed in the nervous system was sufficient for NP hypersecretion. SGK-1 activity responds to three signaling activities: (1) Phosphatidylinositol-3-phosphate (PI3P) signaling, (2) Insulin/Insulin-like growth factor-1 signaling (IIS) as well as (3) target of rapamycin complex-2 (*TORc2*) signaling. Intriguingly, SGK-1 in response to upstream pathways functions as a gate that only is activated upon synergistic signaling from all three activities. Active SGK-1 inhibits the transcription factor DAF-16/FOXO, whereas the activity of DAF-16/FOXO suppressed neuropeptide secretion. Additionally, genetic epistasis analysis suggested that SGK-1 also controls the activity of a PQM-1-CEH-60/PBX-UNC-62/MEIS transcription factor complex with antagonistic activities to DAF-16/FOXO in facilitating NP secretion. In summary, we conclude that SGK-1 is a membrane-bound master-regulator of metabolically controlled neuropeptide release from neuronal cells.

976C **A novel function of *zoo-1/ZO-1* in controlling synapse patterning** Sydney S Ko, Andrew W Snow, Kota Mizumoto Zoology, The University of British Columbia

Zonula occludens (ZO) proteins are a family of tight junction-associated MAGUK (Membrane-Associated Guanylate Kinase) proteins that connect tight junction proteins to the actin cytoskeleton. *C. elegans* possess a single ZO ortholog, *zoo-1*, which has been shown to function at the adherens junction during embryonic development (Lockwood et al., 2008. PMID: 18718757).

While several MAGUK proteins, such as PSD-95 and Discs Large, are crucial for synapse development and function, the functions of ZO proteins at synapses are not understood. Using the NATF (Native and Tissue-Specific Fluorescence) method, we visualized the subcellular localization of endogenous ZOO-1 in the DA9 cholinergic motor neuron and found that ZOO-1::7×GFP was exclusively localized at the axonal region, defined as the synaptic domain, where DA9 forms en passant synapses. Consistent with the synaptic localization of ZOO-1, we found that *zoo-1(tm4133)* loss of function mutants exhibited a tendency to have increased synaptic domain lengths and anteriorly shifted synaptic domains, suggesting that *zoo-1* may function to restrict the synaptic domain. Previous studies have shown that the synaptic domain of DA9 is defined by a series of pro- and anti-synaptogenic signaling molecules, including NRX-1/Neurexin, PLX-1/Plexin, LIN-17/Frizzled, and UNC-6/Netrin. Given that ZOO-1 has three PDZ domains and Neurexin has a PDZ-binding motif, we first explored the potential interaction between *zoo-1* and *nrx-1*. We found that *zoo-1* suppressed the reduced synaptic domain length observed in a null mutant of Neurexin, *nrx-1(miz252)*, in which the entire coding sequence of *nrx-1* is deleted. Interestingly, the *zoo-1(tm4133)* mutant did not suppress the reduced synaptic domain length of *vab-8/KIF26*, which was recently shown to function downstream of *nrx-1* and *mig-1/Frizzled* (Balseiro-Gòmez et al., 2022. PMID: 35809561). This suggests that *zoo-1* may function upstream of *vab-8*. Currently, we are investigating the mechanisms of ZOO-1 localization at the synaptic region and the potential function of *zoo-1* as a regulator of the synaptic domain by exploring its interactions with other molecules involved in DA9 synapse patterning.

977C **Investigating the role of the *ceh-43* homeobox gene in *Caenorhabditis elegans* neuronal specification** James Lao, Berta Vidal, Oliver Hobert Biological Sciences, Columbia University

Terminal differentiation of neuron classes is regulated by homeodomain transcription factors, encoded by highly conserved homeobox genes across animal nervous systems. In *Caenorhabditis elegans*, each neuron class is defined by the expression of a unique combination of homeodomain proteins. Currently, 113 out of the 118 neuron classes of *C. elegans* are known to require a homeobox gene for proper terminal differentiation. Yet, there remain five neuron classes, including the ASJ sensory neuron pair, which do not have a known homeobox regulator. The Distalless/DLX ortholog *ceh-43* is expressed in ASJ and several other neuron classes in the head, midbody, and tail (ADE, AIN, AIZ, ASJ, BDU, CAN, CEP, IL1, PDE, PVQ, SDQ). We previously showed that *ceh-43* is required for the differentiation of dopaminergic neurons and the ring interneuron AIZ, and we are now investigating potential additional roles of *ceh-43* in ASJ and other neurons. To circumvent the embryonic lethality of *ceh-43* null mutants, we generate novel cis-regulatory alleles of the *ceh-43* locus, seeking to selectively eliminate *ceh-43* expression in ASJ and/or other neuron classes in otherwise viable animals. We present here a cis-regulatory mutant analysis of *ceh-43* as a means of finding a homeobox regulator for ASJ, as well as a tool to better study *ceh-43* gene function in other *ceh-43* positive neurons that have not yet been studied for an involvement of *ceh-43* (e.g. SDQ). Comprehensive analysis of homeobox function may point to an evolutionary and central role of this gene family in delineating neuronal identity specification during development.

978C **The molecular and neural regulation of ultraviolet light phototaxis and its food-associated learning behavioral plasticity in *C. elegans*** Kazuki Ozawa[1], Yoichi Shinkai[2], Koichiro Kako[3,4], Akiyoshi Fukamizu[4], Motomichi Doi[21]School of Life and Environmental Science, University of Tsukuba, [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), [3]Faculty of Life and Environmental Sciences, University of Tsukuba, [4]Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance, University of Tsukuba

Caenorhabditis. elegans represents a broad behavioral plasticity in a lot of learning paradigms. Ultraviolet light (UV) is quite toxic to all the animals and evoke the avoidance behavior of UV. *C. elegans* senses UV and is known to avoid UV by using four sensory neurons (ASJ, ASK, AWB, ASH), Because worms do not show avoidance behavior of UV when all these four neurons are simultaneously lost. However, it is not clear what signaling molecules act for UV avoidance in the neuronal pathway constituted of four sensory neurons. In addition, it is not clear whether this harmful environmental signal can be associated with other benefit signals such as food. In this study, by using newly developed assay system in which worms were exposed to UV (350 nm, 5µW/mm2) with food, the avoidance behavior of UV after conditioning was examined. We found that worms strongly avoid UV, but conditioning with UV and food significantly reduced excessive avoidance of UV. This means that *C. elegans* can associate UV and food to change behavioral strategy against harmful UV signal. This is the first indication that *C. elegans* shows associate learning with UV and food.

Using our assay system, we also found that glutamate is used as a transmitter for both the UV avoidance and UV associative learning, whereas different sets of glutamate receptors seemed to act for UV avoidance and UV associative learning (*mgl-1* and/or *mgl-2*, *glc-3* for UV avoidance, *glc-3*, *glr-1* and *nmr-1* for UV learning). To further elucidate the mechanism underlying UV associative learning, we characterized the function of four sensory neurons important for phototaxis. Among these sensory neurons, our analyses suggest that ASH sensory neuron is indispensable for UV associative learning, whereas ASK sensory neuron is not seemed to be required. However, worms that lost ASH and ASK sensory neuron could not change the avoidance behavior after the conditioning with UV and food. These results suggest that another neuronal control system is operating that rewrites instinctive avoidance behavior from harmful UV light.

979C **Benzaldehyde/Starvation Learning Produces a Non-Canonical Memory Type** Alexandra Udachina[1,2], Daniel M. Merritt[3,4], Ninon Freidel[1,2], Sylvia M.T. Almeida[5], Yan Ming Anson Lau[5,6], Derek van der Kooy[3,51]Human Biology Program, University of Toronto, [2]Department of Psychology, University of Toronto, [3]Institute of Medical Science, University of Toronto, [4]Department of Biological Sciences, Columbia University, [5]Department of Molecular Genetics, University of Toronto, [6]Institute of Biomedical Engineering, University of Toronto

Across many animal species, formation of long-term memories requires repeated, spaced training trials (spaced training), as well as de novo transcription and protein synthesis. Single, continuous training sessions (massed training) typically produce protein synthesis-independent, short-lasting memories. The nematode C. elegans is naively attracted to the volatile odorant benzaldehyde; after one hour of exposure to benzaldehyde in the context of starvation, worms switch their behavioral response to a point source of dilute benzaldehyde from approach to avoidance. Through the use of pharmacological inhibitors, we find that despite arising from a massed training paradigm, this form of learning depends on transcription and translation. Further, we show that protein synthesis-dependent memory consolidation continues after the removal of conditioning stimuli, but that protein synthesis is not required for memory recall. While CREB is the canonical regulator of learning-related transcription across multiple species and paradigms, we find that the transcription required for learning in our paradigm is not regulated by either of the C. elegans CREB proteins. In addition, we show that while benzaldehyde/starvation learning is dependent on the insulin signaling pathway at least as far as the kinase AKT-1, loss of the transcription factors acting immediately downstream of AKT-1, DAF-16 and PQM-1, does not mimic the effects of transcription inhibition. Our data provide an example of transcription-dependent memory that does not require CREB and is independent of the DAF-16 output of insulin signaling, highlighting the diversity of ways by which memory can arise depending on the organism and paradigm.

980C **The role of insulin/IGF-1 signaling in modulating *C. elegans* feeding behavior under adverse conditions** Zion Stephanie Walker[1], Surojit Sural[2], Oliver Hobert[21]Biological Sciences, Columbia University, [2]Department of Biological Sciences, Columbia University, Howard Hughes Medical Institute

One of the main factors enabling organismal survival under conditions of adversity is the plasticity of its nervous system. In response to prolonged exposure to environments with limited food, the nematode *C. elegans* alters its developmental trajectory to arrest in the dauer stage, giving rise to new morphology and stage-specific behaviors. The insulin signaling-responsive transcription factor DAF-16/FoxO is involved in cell autonomous and non-cell autonomous dauer remodeling of different tissues, including the remodeling of the pharyngeal nervous system that results in downregulation of pharyngeal pumping. While DAF-16 is known to be involved in interorgan coordination of dauer remodeling between the intestine and pharyngeal nervous system, the mechanism of this signaling axis remains unknown. Through the generation of transgenic worms with constitutive expression of insulin genes in their intestine, we aim to identify insulin peptides secreted from the intestine acting as agonists or antagonists of the insulin receptor DAF-2 to remodel the pharyngeal nervous system under adverse environmental conditions. Based on relative expression in dauer from RNA-seq and gfp promoter fusion data, 18 of the 39 *C. elegans* insulins may act as intestinally produced antagonists or agonists of the DAF-2 receptor. We hypothesized that for agonist insulins that are downregulated under limited food conditions, reduced intestinal insulin secretion decreases activation of the neuronal DAF-2 pathway and increases active DAF-16, which should alter gene expression to silence pharyngeal pumping behavior. Alternatively, insulins released from the intestine might act as antagonists to decrease DAF-2 signaling and allow for dauer remodeling of the pharynx. We find that constitutive expression of combinations of nine potential agonist insulins in the intestine had no effect on the cessation of pumping in the dauer pharynx. In contrast, constitutive expression of a single candidate antagonist insulin in the intestine reduces the pharyngeal pumping of adult animals, suggesting that this peptide may act as an antagonist to DAF-2 regulating DAF-16 activity. We are currently investigating whether deletion of this candidate antagonist insulin, either alone or in combination with others, can reverse the silenced state of the dauer pharynx. Ultimately, this work aims to elucidate interorgan insulin signaling dynamics that remodels the *C. elegans* pharyngeal nervous system and alters feeding behavior under adversity.

981C **Genus-specific duplication of PRD/OTX homeodomain transcription factors in *Pristionchus pacificus*** Dylan L Castro, Svetlana Kuznyetsova, Ray L Hong Biology, California State University Northridge

Homeodomain proteins are important for specifying neuronal identity across taxa. In *C. elegans*, the PRD/OTX class of homeodomain transcription factors, *ceh-37, ceh-36,* and *ttx-1* regulate the development and maintenance of several amphid neurons. However, in *Pristionchus pacificus*, a predatory entomophilic nematode, *ceh-36* and *ceh-37* lack clear 1-1 homology, and comparisons with other nematode genomes suggest a likely duplication in the *Ppa-ceh-36* homolog. Since other homeodomain genes share 1-1 orthology between these two species, the presence of *Ppa-ceh-36.1* and *Ppa-ceh-36.2* represents a rare case for studying how nascent paralogs can be incorporated into a homeodomain gene code for neuronal specification. We found that transcriptional fusion reporters show significant differences in expression patterns when compared to their *C. elegans* counterparts. *Cel-ceh-36* and *Cel-ceh-37* are expressed in the AWA, AWB, AWC, ASI, and ASE amphid neurons. While only *Ppa-ceh-37p::gfp* shows expression in several pairs of amphid neurons, *Ppa-ceh-36.1* and *Ppa-ceh-36.*2 promoter reporter fusions are expressed in the

support glia, such as the amphid and phasmid sheath cells, and pharyngeal neurons and gland cells, respectively. Moreover, we found that the *Ppa-ceh-36* duplication is unique to the *Pristionchus* genus and not found in the genomes of other members of *Diplogasteridae* such as *Allodiplogaster sudhausi* and *Micoletzkya japonica,* nor more distantly related nematodes such as *C. angaria* and *Strongyloides stercoralis*. We are currently studying how mutations in *Ppa-ceh-37* and *Ppa-ttx-1* can affect amphid neuron specification as well as chemotactic and thermotactic behaviors.

982C **Roles of the monoamine transporter CAT-1 in *Pristionchus pacificus*** Megan F Hampton[1], Heather R Carstensen[1], Curtis M Loer[2], Ray L Hong[11]Biology, California State University, Northridge, [2]University of San Diego

Despite roughly ~200 million years of independent evolution and significant changes in development and behavior, *Pristionchus pacificus* and *C. elegans* have almost the same number of neurons. One possible contributor to changes in neuronal function could come from changes in the regulation of neurotransmitter types in homologous neurons, such as through alterations in the expression of phylogenetically conserved proteins involved in monoamine synthesis and transport. Specifically, serotonin signaling is required for egg-laying in both species, but serotonin is also involved in the predatory behavior particular to *Pristionchus* species. To identify potential roles of monoaminergic neurons, we used CRISPR/Cas9 to produce two loss-of-function mutants in *Ppa-cat-1,* which encodes a vesicular monoamine transporter for serotonin, dopamine, tyramine, octopamine, and the non-classical neurotransmitter betaine. Serotonergic neurons shared between *P. pacificus* and *C. elegans* include the pharyngeal NSMs, amphidial ADFs, putative RIM, and 4 vulval region VCs (likely involved in egg-laying). There are also at least two pairs of 5HT neurons particular to *P. pacificus*- the pharyngeal I1s, and the pair of strongly staining RIP neurons connecting the somatic and pharyngeal nervous systems that are not serotonergic in *C. elegans.* We found that *Ppa-cat-1* mutants have a slow egg-laying phenotype (egl) that leads to egg accumulation in the uterus, which can be rescued with the addition of exogenous serotonin and thus validating a conserved role for serotonin in egg-laying. *Ppa-cat-1* mutants also have other behavioral phenotypes: *Ppa-cat-1* dauer larvae are unable to nictate (standing and waving), while *Ppa-cat-1* J4 larvae show slight hypersensitivity to exogenous betaine in paralysis assays. We additionally made a *Ppa-cat-1p::GFP* reporter strain and found expression in the known 5HT neurons and dopaminergic CEP and PDE neurons. However, we did not detect reporter expression in the CAN neurons or cells that correspond to the HSN homologs, consistent with our anti-5HT staining result. Using this *Ppa-cat-1p::GFP* transcriptional reporter, we aim to identify potential transcriptional regulators of *Ppa-cat-1* and their possible role in the divergent expression of this monoamine transporter.

983C **GNAO1, Sleep, and Insomnia: Humanization of *C. elegans goa-1*** Jacqueline S Cho[1], Adam Friedberg[2], Anne C Hart-[11]Neuroscience, Brown University, [2]Brown University

Sleep is an essential behavior observed across species and is characterized by behavioral quiescence, including reduced sensory responsiveness and decreased activity. In *C. elegans*, Notch signaling regulates developmentally timed sleep (DTS) which occurs just before molting during periods known as lethargus. A double knock-out of the two Notch co-ligands (osm-7 and osm-11) leads to a decrease in DTS, whereas overexpression of OSM-11 in adult *C. elegans* results in anachronistic sleep, a temporary, reversible behavioral quiescence similar to lethargus sleep (Singh 2011). With this knowledge, forward genetics was used to screen for genes whose perturbation disrupted anachronistic sleep in *C. elegans*, and *goa-1*, a gene that encodes a G-alpha(o), was identified (Huang 2017). Notably, the orthologous human gene GNAO1 was also found to be significantly associated with insomnia in a human genome wide association study, suggesting GNAO1 may have a potential regulatory role in sleep across species (Lee 2019). To investigate this, we will humanize the *C. elegans* genomic background using a two-phase approach by (1) removing all coding sequences of *goa-1* and inserting an optimized sequence encoding GNAO1 and then (2) iteratively knocking-in introns of GNAO1. We will assess the viability of whole-gene humanization and we will test conservation of function using anachronistic sleep and DTS assays relative to controls. If humanization is successful, we will use CRISPR-Cas9 to knock-in GNAO1 missense mutations identified in human insomnia patients to study GNAO1 disruption in *C. elegans*. Leveraging CRISPR-Cas9 technologies and population-genetics studies, we aim to demonstrate conservation of GNAO1 in *C. elegans*, investigate mechanistic contributions of insomnia-associated regions of the chromosome, and identify novel contributions of GPCR signaling in sleep and insomnia.

984C **A serotonergic pathway mediating the effects of gut microbes on host feeding behavior** Dina Garmroudi, Madhumanti Dasgupta, Michael O›DonnellMolecular, Cellular, and Developmental Biology, Yale University

The gut-brain axis can provide key insights into processes such brain development and neurogenesis, but also behavioral disorders and neurodegenerative disease. The nervous system is not an isolated system—microbes colonizing the gut may regulate neurons via the production of neuroactive compounds. For example, gut microbiota can produce neurotransmitters, some of which may alter animal behavior. Microbiome-depleted or gnotobiotic organisms can help to highlight the impact of gut microbiota on host behavior, and this approach has been employed in diverse animals to identify neuronal traits that depend on the gut microbiome. There is evidence in animals that gut microbiota can impact host feeding decisions, which in certain circum-

stances may benefit resident gut microbes. However, the molecular mechanisms by which gut microbiota impact host feeding behavior are not well understood. This is partly because the mammalian nervous system, intestine, and gut microbiome are complex, rendering the identification of chemicals involved in these effects challenging. *Caenorhabditis elegan*s is an excellent system to study how the gut microbiome impacts feeding due to its short generation time, a microbiome that can be easily manipulated with gnotobiotic culture techniques, a fully mapped neuronal connectome, and amenability to genetic manipulation. We are interested in determining if and how naturally occurring gut commensals impact the rate of feeding in *C. elegans*. Pharyngeal pumping is a simple measure of feeding behavior in *C. elegans.* Serotonin regulates pharyngeal pumping via multiple mechanisms. When wild-type worms are colonized by *Providencia*, a naturally occurring gut commensal for *C. elegans*, feeding behavior is largely unchanged compared to worms fed laboratory *E. coli* strains. However, mutations in *ser-7,* which encodes a conserved serotonin receptor, result in reduced feeding rates specifically when colonized by *Providencia*. We hypothesize that *Providencia* either causes the worm to produce more serotonin or produces a serotonin receptor agonist. This increased serotonin signaling in the absence of SER-7 function then results in reduced feeding rates. We hypothesize that gut microbial influences on serotonin signaling may potentiate specific neuronal pathways to influence feeding. Better characterizing the role of gut microbiota in feeding will inform how essential behaviors emerge and are impacted by animals' internal and external environments.

985C **A *C. elegans* digital twin** Yunjie J Zhu[1], Christopher A Brittin[2], Netta Cohen[11]School of Computing, University of Leeds, [2]Developmental Biology Program, Memorial Sloan Kettering Cancer Center

Brain function emerges through the structural organization of synaptic contacts. The *C. elegans* nerve ring exhibits structural patterns of highly distributed synaptic connectivity [1] and functional patterns of coordinated neuronal activity [2]. To better understand the structure-function relation in the nerve ring, we developed a model framework for simulating whole-brain activity based on known synaptic contacts in the *C. elegans* nerve ring. The network is modelled as a continuous-time recurrent neural network with identical neurons. The default network connectivity is drawn from the conserved reference connectome (C4 in [1]). Synaptic weights and polarities in our model are optimized using a deep artificial neural network (training the biological model such that the model neuronal circuit dynamics closely match whole-brain imaging recordings. Our framework identifies a family of 30 large-scale computational models that closely match the recording of nerve ring dynamics in behaving animals [2]. Analysis of these models points to general patterns of synaptic strengths and polarity that cannot be inferred directly from anatomical data. We find conditions for persistent oscillations and a minimally required fraction of inhibitory synapses. Our models predict how network dynamics change under different perturbations and manipulations. Our experiments demonstrate the promise of whole-brain computational models for linking anatomically derived structural data and functional recordings of neuronal activity.

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[2] S. Kato et al. (2015). Global brain dynamics embed the motor command sequence of Caenorhabditis elegans, Cell 163:656–669.

986C **Uncovering new GABA transporters in *C. elegans* thanks to an atlas of amino acid transporter expression** Louna Schaison[1], Nalia Samba[1], Elise Cheynet[2], Charlotte Tissot[1], Marie Gendrel[11]Biology, IBENS - ENS - INSERM - CNRS - PSL University, [2]MeLis, CNRS UMR 5284, Univ Claude Bernard Lyon 1

Functional neuronal circuits rely on a combination of both excitation and inhibition. In mature neurons, the main inhibitory neurotransmitter is GABA. Traditionally, neurons have been classified as GABAergic if they co-expressed three protein determinants: 1) GAD/UNC-25, which synthesizes GABA from glutamate, 2) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and 3) GAT/SNF-11, a plasma membrane transporter that recaptures extracellular GABA. In *C. elegans*, only 26 out of 302 neurons were considered GABAergic, until an improved immunostaining enabled the identification of 15 additional GABA-positive neurons. Although they stain for GABA and contact neurons expressing post synaptic $GABA_A$ receptors, they do not all co-express GAD/*unc-25*, *VGAT/unc-47* and GAT/*snf-11*. In particular, three pairs of neurons express none of those. Thus, they are unable to synthesize, uptake or package GABA the way we know it.

We hypothesize that those neurons use unidentified transporters for GABA uptake and vesicular packaging. The present work aims to identify them, and in particular to probe 53 putative amino acid transporters belonging to the SLC6 (GAT/SNF-11's family), SLC7/3, SLC16 and SLC36/38 families. Most of these transporters are not functionally characterized yet. Thus, as a first step, we undertook to determine the expression pattern of the candidates. So far, using a fosmid-based reporter strategy (Tursun *et al*, 2009), we generated transgenic lines for 19 genes out of the 53 putative amino acid transporters in order to establish an atlas of amino acid transporter expression. To note, neuronal expression could be detected in 11 out of the 19 genes and,

thanks to anti-GABA staining, we could assess that at least three of them were express in our three pairs of neurons of interest.

The completion of the atlas is in progress and, thanks to the NeuroPAL strain (Yemini *et al*. 2021) and other cell specific markers, a precise map of the expression sites of the 53 amino-acid transporter will be established. Moreover, for the genes expressed in our neurons using unknown alternative GABA transporters, we will generate CRISPR KO lines in order to assess their role in GABA transport.

987C **A refined, high-threshold evaluation of sex-specific synapses in the *C. elegans* nerve ring** Cristine A Kalinski, Steven J Cook, Oliver Hobert Biological Sciences, Columbia University

The nervous system of *C. elegans* is sexualized for both reproductive and sex-specific behaviors with associated dimorphisms in neuron number, morphology, and connectivity. Connectomes created using serial section EM have described the nanoscale features of the nervous systems of both sexes, and comparisons have revealed widespread differences in connectivity among sex-shared neurons (Cook et al., 2019, *Nature*). The recent publication of additional hermaphrodite connectomes has allowed for extensive study and characterization of the significant variability in connectivity between individual animals of the same-sex, motivating us to re-examine the sexually dimorphic connectivity of the *C. elegans* nerve ring (Witvliet et al., 2021, *Nature*; Cook et al, 2022, *bioRxiv*). We thresholded sex-specificity such that a connection must be present in all samples of one sex but in zero of the other (hermaphrodite n=10, male n=1). This criteria allows for stricter classification of sexual dimorphism in connectivity within the context of inter-individual variability. Indeed, we find that the majority of connections previously classified as sexually dimorphic are the products of inter-individual variability. Our analysis shows that 5.9% of nerve ring connections are sexually dimorphic, and that these connections are broadly distributed across 83.8% of the sex-shared neuron classes. Furthermore, using the nine available hermaphrodite 'contactomes', we find that the hermaphrodite-specific connections were associated with stronger surface-area contact, on average, while male-specific connections were associated with smaller, more variable contacts. Our analysis provides a valuable resource for future explorations of the genetic underpinning and functional consequences of sexual dimorphisms in the brain.

988C **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 Zoology, 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 could 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 DB class of cholinergic motor neurons using the recCaspase-3 system resulted in a moderate but significant synaptic tiling effects 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 found that DB ablation (recCaspase-3 or *vab-7*) enhanced the synaptic tiling defect of *plx-1(nc36)/PlexinA*, suggesting that the DB7 axon does not act through Plexin signaling to control DA8-DA9 synaptic tiling. We conducted a candidate screening to identify cell adhesion and signaling molecules mediating DA-DB interaction and found a null mutant of *syg-2(miz185)*, which has a nonsense mutation in exon 12, that exhibited a moderate synaptic tiling defect between DA8 and DA9 similar to *vab-7(e1562)* mutants. Interestingly, a *syg-2* mutant lacking its cytoplasmic domain also displayed synaptic tiling defects between DA8 and DA9, suggesting that SYG-2 signals through its cytoplasmic domain to control DA8-DA9 synaptic tiling. Currently, we are continuing to investigate the role of *syg-2* in synaptic tiling as well as investigating its known binding partner, *syg-1*.

989C ***In vivo* functional study of an alternative protein encoded by the gene *ZYX/zyx-1* with implications for synaptic development and dystrophinopathies** Noémie Frébault[1], Benoît Grondin[2], Lise Rivollet[1], Benoît Vanderperre[1], Claire Bénard[1,31]Dept. Biological Science, CERMO-FC Research Center, Université du Québec à Montréal, [2]Dept. Biological Science, CERMO-FC Research Center, Université de Québec à Montréal, [3]Dept. Neurobiology, University of Massachusetts Chan Medical School

During development, neurons and muscles establish precise morphologies to ensure specific functions, which need to be subsequently maintained throughout life. Yet, the mechanisms underlying their lifelong maintenance are not well understood. Defective synapse maintenance and muscle degeneration are involved in Duchenne muscular dystrophy, which is caused by a deficiency in the sarcolemmal protein dystrophin. The gene *ZYX* encodes Zyxin, a cell adhesion and mechanosensation protein. Its *C. elegans* homolog, *zyx-1*, is required for the development of synapse and neuronal branches, as well as for body wall mus-

cle maintenance against dystrophin-dependent degeneration. Indeed, either overexpression or deficiency of *zyx-1* ameliorates the muscle degeneration phenotype caused by dystrophin deficiency. In addition to encoding the canonical protein Zyxin, the gene *ZYX/zyx-1* codes for a second distinct protein named AltZyxin produced from an alternative open reading frame (altORF), as revealed by mass spectrometry studies in both humans and *C. elegans*. Some altORFs-encoded proteins have been found to physically interact with the canonical protein encoded by the same gene, to modulate their cellular localization and molecular function, and to regulate their expression. To decipher the mechanism by which the gene *ZYX/zyx-1* regulates synaptic and muscular maintenance and degeneration, we are investigating the function of the alternative protein AltZyxin in synapse and muscle biology in *C. elegans.* For this, we generated CRISPR-engineered targeted mutations to specifically disrupt either of the two encoded proteins, and determine the synaptic, muscle, and behavioral consequences. Neuron- and muscle-specific rescue assays and localization studies with fluorescent reporters will inform on the functions and interplay between Zyxin and AltZyxin. Finally, *in vivo* TurboID screening is being prepared to identify AltZyxin interactors, which may provide insight into AltZyxin's functions. We expect that our studies of the recently identified *zyx-1/ZYX* product AltZyxin on maintenance processes will provide a better understanding of mechanisms involved in Duchenne muscular dystrophy and neurodegenerative diseases.

990C **Pathogen infection induces sickness behaviors by reconfiguring the neuropeptide systems that control *C. elegans* sleep** Sreeparna Pradhan, Gurrein K Madan, Jungyeon Park, Ugur Dag, Matthew A Gomes, Steven W Flavell MIT

Defensive responses to pathogen infection are essential for host organism recovery and survival. In the nematode *C. elegans*, infection by the human opportunistic pathogen *Pseudomonas aeruginosa* strain PA14 leads to the activation of multi-level defenses, including innate immune signaling pathways and dramatic behavioral changes. However, how infection impacts brain function to trigger defensive behaviors remains mostly unknown. Here, we show that PA14 infection induces a reconfiguration of the neuropeptidergic systems that control *C. elegans* sleep, which allows animals to generate sickness behaviors that defend against pathogen infection. Specifically, we found that the neuropeptide FLP-13 and its cognate G-protein coupled receptor DMSR-1, which are required for stress-induced-sleep in uninfected animals, are functionally reconfigured upon infection to be required for wakefulness. Infected animals lacking *flp-13* or *dmsr-1* display PA14-induced quiescence, characterized by an absence of feeding and locomotion, and subsequently die as a result of it. We show that such quiescence requires active pathogen infection and is caused by specific virulence factors released by *P. aeruginosa*. Genetics, optogenetics and time-course assays reveal this quiescence is reversible, and recovery from quiescence increases the survival rate of infected *flp-13* and *dmsr-1* mutants. Through cell-specific manipulations and calcium imaging, we identify the exact neurons that act as critical sources of FLP-13 in infected animals, which do not include the ALA neuron that releases FLP-13 to promote sleep in uninfected animals. Through brain-wide calcium imaging, we characterize the PA14-induced quiescence brain state displayed by *flp-13* mutants. Overall, these results suggest a model where specific sleep-associated neural pathways are functionally repurposed upon gut infection to promote sickness behaviors. State-dependent changes in the functions of neuropeptidergic systems, like the one described here, may endow nervous systems with a previously unappreciated level of flexibility.

991C **A ventral source of UNC-6/Netrin is not required for dorsal-ventral axon guidance** Kelsey Hooper[1], Snehal Mahadik[2], Erik Lundquist[21]Molecular Biosciences, University of Kansas, [2]University of Kansas

The commissural growth of the VD/DD axons is controlled by the guidance cue UNC-6/Netrin, which is expressed in the ventral nerve cord (VNC). VD growth cones migrate dorsally away from the UNC-6/Netrin source (i.e. repulsion). Classically, commissural axon migration in response to UNC-6/Netrin was thought to occur in response to a ventral-to-dorsal concentration gradient emanating from the VNC. Growth cones of VD/DD axons were thought to dynamically interpret this gradient. Previous growth cone imaging studies in our lab indicate that this mechanism is more complex, involving discrete aspects of growth cone polarity coupled with regulation of filopodial protrusion (the Polarity/Protrusion model). The VD growth cones contain two UNC-6 receptors: UNC-5 and UNC-40. The UNC-5 receptor initially polarizes the growth cone, establishing a dorsal bias of filopodial protrusion and actin accumulation. After polarization, UNC-5 inhibits protrusion on the ventral side of the growth cone while UNC-40 stimulates protrusion on the dorsal. This creates a dorsal asymmetry of protrusion which drives dorsal migration of the axon. To further test this model, we created a transmembrane UNC-6, *unc-6(lq154)*, which is predicted to eliminate diffusible UNC-6. *unc-6(lq154)* fails to guide the axons of the AVM and PVM neurons, which are dependent on the presence of diffusible UNC-6. In *unc-6(lq154)* mutants, VD growth cones were initially polarized, but lost polarity as they migrated away from the VNC. Thus, a close-range interaction polarizes the growth cone, and maintenance of polarity during continuous migration beyond the VNC may require non-directional diffusible UNC-6. To test this idea, wild-type diffusible *unc-6* was expressed from anterior (pharynx) and dorsal (body wall muscle) sources. Both rescued growth cone polarity defects of *unc-6(lq154)* and, surprisingly, *unc-6(ev400)* null mutants. Ectopic expression of diffusible UNC-6 was also sufficient to guide the ventrally directed AVM and PVM axons in both *unc-6(lq154)* and *unc-6(ev400)*. In sum, these data suggest that a ventral directional source of UNC-6 is not required for dorsal VD or ventral AVM/PVM axon guidance, and that UNC-6 might be a permissive, rather than an instructive, cue in axon guidance. Possibly, neurons have an inherent dorsal-ventral polarity. Alternatively, UNC-6 might function with another

directional cue, both being required but only one directionally.

992C **Using the million mutation project strains to identify genes regulating sleep** Kerry M LeCure, Aylin Ergin, David RaizenUniversity of Pennsylvania

C. elegans worms reduce body and feeding movements and increase sleep when exposed to environmental stressors such as heat and UV radiation. The Million Mutation Project (MMP) is a collection of 2007 strains of heavily mutagenized worms; collectively, the strains contain a loss-of-function mutation of almost every non-essential *C. elegans* gene. Each strain has been fully sequenced and cataloged. To find sleep-regulation genes, we are screening MMP strains using WorMotels. After stressing worms with UVC light, we measure movement quiescence using machine vision. When we find a strain with a defect in SIS, we do balancer mapping in order to identify the chromosome containing the causal gene variant, and thus identify candidate genes mediating the phenotype. We confirm genotype to phenotype causality by testing additional loss-of-function alleles of the gene of interest. So far, we have screened ~900 MMP strains and have identified ~15 strains with total quiescence that is at least 2 standard deviations lower than the global mean of all strains. In support of our approach, one strain with an SIS-defective phenotype contains a loss-of-function mutation in *ceh-14*, a gene known to function in SIS. We will report an update on our screen, including the identification of the conserved ubiquitin ligase *eel-1* as required for SIS.

993C **A pan-neuronal alternative splicing event triggers pan-neuronal gene transcription** Eduardo Leyva Diaz[1,2], Michael Cesar[2], Oliver Hobert[21]Instituto de Neurociencias, CSIC-UMH, [2]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University

Gene expression programs in differentiating neurons can be subdivided into at least two types, those that control expression of neuron type-specific proteins and those that control expression of proteins that are shared by all cells in a nervous system, such as proteins involved in the synaptic vesicle cycle or in neuropeptide biogenesis. Pan-neuronal gene expression is controlled by members of the CUT homeobox gene family, including the pan-neuronally expressed *ceh-44*. We address here how the expression of *ceh-44* is directed to the nervous system. Our studies show that *ceh-44* pan-neuronal expression is triggered by a pan-neuronal RNA splicing factor, UNC-75, the *C. elegans* homolog of vertebrate CELF proteins. UNC-75 spatially specifies the production of an alternative, CEH-44 homeobox gene-encoding transcript from a ubiquitously expressed gene locus, which can also produce a conserved Golgi apparatus-localized Golgin protein, CONE-1 ("**C**ASP **o**f **ne**matodes"). During embryogenesis, before terminal tissue differentiation, the CONE-1/CEH-44 locus exclusively produces the Golgi-localized CONE-1 protein in all tissues, but upon the onset of postmitotic terminal differentiation of neurons, UNC-75 binds to the CONE-1/CEH-44 transcript to redirect the splicing machinery to now produce the alternative, CEH-44 CUT homeobox gene-encoding transcript, exclusively in the nervous system. CEH-44 subsequently controls the expression of pan-neuronal effectors genes, such as proteins of the synaptic vesicle cycle. Hence, UNC-75-mediated alternative splicing not only directs pan-neuronal gene expression, but also excludes a phylogenetically deeply conserved Golgin from the nervous system, paralleling surprising temporal and spatial specificities of other Golgins that we describe here as well. Remarkably, the unusual combination of Golgin and homeobox gene production from a single locus is conserved in vertebrates as well. Our findings provide novel insights into how all cells in a nervous system acquire pan-neuronal identity features.

994C **Investigating the Intersection of the Dopaminergic Circuit with a Blue-Light Sensing Circuit** Nyaluak Gayluak Fisk University

Dopamine is a neurotransmitter important for motor control and cognitive function1 and is associated with feelings of pleasure, motivation, and sense of reward. Several diseases are associated with dysregulation of dopamine levels, such as Parkinson's disease (PD), schizophrenia, and attention deficit hyperactivity disorder (ADHD). However, multiple neural circuits are likely to intersect and integrate with the dopaminergic pathway and may be able to impact downstream signaling. The goal of this research project is to better understand the intersection of the dopaminergic circuit with a blue-light sensing circuit in the model organism *Caenorhabditis elegans* (*C. elegans*). In *C. elegans*, an accumulation of dopamine leads to the inhibition of motor activity and therefore paralysis. In a swimming-induced paralysis assay (SWIP), worms lacking the gene encoding the dopamine transporter (*dat-1* mutants) paralyze rapidly due to accumulation of extrasynaptic dopamine. However, we have observed that when paralyzed worms are exposed to blue light they are reanimated, suggesting that the blue light signaling pathway overrides the inhibition of motor neurons by extrasynaptic dopamine. Previous work by others has shown that worms possess neural circuitry for avoiding blue light. We postulate that the neural circuit activation specifically for evading blue light can override paralysis caused by extrasynaptic dopamine. We will look at worms that lack a key gene (*lite-1*) for blue light avoidance.The findings from this research may contribute further understanding of control of neural circuits, including those associated with dysregulation of dopamine levels, by examining the various neural circuits that intersect and integrate with the dopaminergic pathway.

995C ***CelegansWholeIntegration*: An Open-Source Simulation Platform for *C. elegans* Nervous System and Body** Jimin Kim[1], Linh Truong[1], Eli Shlizerman[21]ECE, University of Washington, Seattle, [2]ECE, AMATH, University of Washington, Seattle

We present an open-source simulation platform called *CelegansWholeIntegration*, for the computational studies of *C. elegans* nervous system and body. The platform allows for the simulation of the whole somatic nervous system and its response to stimuli and connects it with body dynamics. The platform features a model incorporating full anatomical wiring diagram, somatic connectome, intracellular and extracellular neural dynamics. The model also includes layers which translate neural activity to muscle forces and muscle impulses to body postures. In addition, it implements inverse integration which modulates neural dynamics according to external forces on the body, emulating proprioceptive feedback. Here we test the model by recapitulating outcomes of body dynamics with various *in-vivo* experiments of the touch responses and implementing neural ablations found effective in them. Furthermore, we use the platform to explore new novel scenarios including connectome modulations and incorporate recently discovered spiking neurons into the model to elucidate novel details on these experiments. The platform also allows for model variations to further enhance its simulated behaviors. As a fully open-source platform, *CelegansWholeIntegration* hosts a public Github repository and an online blog where studied scenarios are described and invite community to identify future novel scenarios.

996C **Rotenone induced dopaminergic neurodegeneration requires sustained neuronal ROS production in *C. elegans*** Katherine Morton, Shefali Bijwadia, Christina Bergemann, Joel MeyerNicholas School of Environment, Duke University

Mitochondrial electron transport chain complex I dysfunction has been implicated in the pathology of Parkinson's Disease (PD). Approximately 80-85% of PD cases are idiopathic, and chronic exposure to mitochondrial toxicants is believed to contribute to disease onset. Rotenone is a pesticide commonly used to model PD and epidemiologically associated with PD. Complex I (CI) inhibition by rotenone results in increased CI superoxide production into the mitochondrial matrix that ultimately leads to neurodegeneration. Though idiopathic PD occurs dominantly in aged individuals, it is unclear how the life stage and length of exposure interact to lead to disease. In *Caenorhabditis elegans*, rotenone exposure is used to model PD by inducing degeneration of the cephalic (CEP) neurons and can be evaluated throughout the lifetime of the worm. We have examined acute and chronic rotenone exposures in developmental, young adult, and aged worms to understand the interactions between aging, rotenone-induced superoxide production, and dopaminergic neurodegeneration. Chronic developmental rotenone exposures from egg to larval stage 4 (L4) only induce dopaminergic neurodegeneration at doses high enough to induce significant developmental delay. In this chronic exposure paradigm, low doses do not induce significant degeneration of the CEP neurons as L4s or day 6 adults; however, they sensitize adults to secondary challenges. Using reduction oxidation sensitive GFP (roGFP) localized to dopaminergic neuronal mitochondria, we find that despite increasing redox stress in the CEP neurons, 4-hour exposure of young adults to 25 uM rotenone is insufficient to induce neurodegeneration immediately or by day 6 of adulthood. However, as day 6 adults, an acute exposure to 25 uM rotenone induces significant neurodegeneration. Together, these data suggest that acute bursts of CI-derived superoxide are not sufficient to induce neurodegeneration until worms are aged significantly. Chronic developmental exposure to low doses of rotenone does not directly induce degeneration immediately or later in life; however, it does sensitize worms to secondary acute exposures. Thus, until worms are significantly aged, induction of dopaminergic neurodegeneration requires sustained reactive oxygen species production within the neurons.

997C ***B. subtilis* induced changes in the *C. elegans* transcriptome that contribute to the protection against α-synuclein aggregation** Samanta Paz Recio[1], Maria Eugenia Goya[1,2], Zoë Hopewell[1], Maria Doitsidou[11]University of Edinburgh, [2]ERIBA, UMCG, University of Groningen

Parkinson's disease is the second most common neurodegenerative disorder worldwide. Its main pathological hallmark is the accumulation of α-synuclein. Growing evidence suggests that the pathology can start in the gut and progress to the central nervous system, and that gut microbiota plays a role in the onset but also in the progression of the disease.

Using a *C. elegans* model that overexpresses human α-synuclein fused to YFP (Van Ham *et al*, 2008) we previously showed that a probiotic bacterial species, *B. subtilis* PXN21, inhibits and reverses aggregation when fed to the worms (Goya *et al*, 2020).

To understand the host response pathways triggered by *B. subtilis* that contribute to this protective effect, we performed comparative transcriptomics analysis on worms fed on the probiotic or *E. coli* OP50 and found over 300 genes upregulated by *B. subtilis*. To find which of the upregulated *C. elegans* genes are involved in the *B. subtilis* mediated protective effect, we performed additional RNA sequencing on worms grown on the wild type *B. subtilis* 168 strain, and compared it to the transcriptome of animals grown on isogenic single gene deletion *B. subtilis* strains that have lost the ability to reduce aggregation. This data helped us compile a list of 26 core genes which are upregulated only when the protective effect is present. These genes point to potentially protective pathways including innate immune responses, redox processes and lipid metabolism.

We also showed that three members of the sphingolipid metabolism pathway, namely *lagr-1, asm-3, and sptl-3,* have roles in the anti-aggregation effect (Goya *et al*, 2020). We are further exploring the role of the sphingolipid pathway by analysing worms lacking beta-glucocerebrosidase (GBA), a lysosomal enzyme that catalyses the conversion of glucocerebroside into a ceramide.

Mutations in the GBA gene in humans are a common risk factor for Parkinson's disease. Therefore, we are generating a quadruple deletion of the four GBA orthologs in *C. elegans*, three of which are upregulated by *B. subtilis*. Furthermore, we are also testing deletions of all 26 upregulated genes for their involvement in inhibiting α-synuclein aggregation.

In a parallel and complementary approach, we are working on uncovering the bacterial signals and metabolites that trigger the protective effect (see poster by Deep Prakash *et al*).

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998C **The neuropeptide receptor *npr-38* regulates avoidance and recovery sleep** Emily Le[1], Caroline Curtin[1], Madison Honer[2], Teagan McCarthy[1], Jonathan Fingerut[1], Matthew Nelson[1]1Biology, Saint Joseph's University, [2]Lewis Katz School of Medicine

C. elegans demonstrates protective avoidance behavior in the face of threats. When exposure to these noxious stressors is unavoidable, cellular damage and injury is incurred. Stress-induced sleep promotes recovery by redistributing resources towards cellular repair. How *C. elegans* coordinates a state of heightened arousal (i.e. avoidance) with that of recovery sleep is unclear. We have identified *npr-38*, a G-coupled protein neuropeptide receptor, as a key component of these dynamics. Loss-of-function of *npr-38* disrupts avoidance and reduces stress-induced sleep. *npr-38* mutants also show reduced activity outside of sleep, evident by reduced spontaneous movement and arousal threshold, but move normally when stimulated with harsh touch. Using cell-specific rescue of *npr-38* and degradation of AID-tagged NPR-38 protein, we identified the ADL sensory neurons as the primary site of action for avoidance and sleep, and the RIS and DVA for arousal. Genetic ablation of the ADL neurons increases sleep, while optogenetic activation reduces it. Multi-copy over-expression of *npr-38* shortens the avoidance phase, resulting in early sleep and premature waking. While the ADL neurons are glutamatergic, we identified *nlp-50* and *flp-25* neuropeptides as being wake-promoting and likely released from the ADL. Based on our data and the connectivity, we propose a model in which the ADL neurons inhibit sleep neurons (i.e., ALA and RIS) to promote heightened arousal during the avoidance phase. *npr-38* then inactivates the ADL neurons to ensure that stress-induced sleep is initiated and terminated at the appropriate times.

999V **Single-cell profiling reveals striking diversity within adult *C. elegans* motor neurons and new functions for a terminal selector gene** Jayson J Smith[1], Seth R Taylor[2], David M Miller[2], Paschalis Kratsios[1]1Department of Neurobiology, University of Chicago, [2]Department of Cell and Developmental Biology, Vanderbilt University

To reveal gene regulatory mechanisms that define neuronal identity and maintenance, we used single-cell RNA-sequencing (scRNA-seq) to profile motor neurons in the adult *C. elegans* ventral nerve cord. We find that eight motor neuron (MN) classes, previously defined by anatomical criteria, subdivide into 29 distinct subclasses delineated by unique Hox and neuropeptide expression codes. Profiling of adult cholinergic MN subclasses lacking the terminal selector *unc-3* (Collier/Olf/EBF), ortholog of the neurodevelopmental syndrome-causing gene *EBF3*, revealed differential responses; eleven subclasses collapse into seven groups, each acquiring new, distinct molecular features, such as alternative neurotransmitter identities. Intriguingly, loss of *unc-3* in cholinergic MNs also disrupts the transcriptomes of post-synaptic GABAergic neurons. Electron microscopy reconstruction suggests that altered gene expression in GABA MNs perturbs connectivity. Thus, unbiased molecular profiling at single-cell resolution uncovered striking diversity within adult MNs, as well as cell-autonomous and indirect mechanisms through which a terminal selector gene sculpts neuronal identity.

1000V ***Caenorhabditis elegans* betaine-sensitive nicotinic receptors: molecular function and physiological roles** Ornella Turani, Guillermina Hernando, Noelia Rodriguez Araujo, Cecilia Bouzat‌I NIBIBB-CONICET-UNS

Caenorhabditis elegans possesses an extensive and diverse family of nicotinic receptors (nAChR), many of which remained uncharacterized. nAChRs are involved in worm locomotion and are targets of anthelmintic drugs. Parasitic nematodes have acquired resistance to most anthelmintic drugs, thus generating problems in human and animal health. Because of this, the identification of novel drugs and targets is required. The potent nematocidal drug monepantel (MNP), which belongs to the recently discovered class of compounds amino-acetonitrile derivatives (AADs), has been shown to target ACR-23 nAChR. ACR-23, whose endogenous agonist is betaine (BE), is a poorly characterized nAChR present in body-wall muscle and mechanosensory neurons of nematodes. Since it is not conserved in vertebrates, ACR-23 is an interesting pharmacological target for anthelmintic drugs. Our goal is to decipher ACR-23 molecular function and its potential as a novel anthelmintic drug target. By performing locomotion assays with wild-type adult worms we showed that exogenous BE significantly increased worm motility. This effect was not observed in *acr-23* mutants, indicating that the hypermotility is mediated by ACR-23. The exposure of worms to MNP produced the opposite effect, resulting in reduced motility as a function of concentration (EC_{50} = 50 µM). MNP induced spastic

paralysis and inhibited egg hatching, indicating important anthelmintic ability. Locomotion assays with mutant worms demonstrated that MNP-induced paralysis is mediated by ACR-23 and DEG-3/DES-2, a nAChR present in sensory neurons involved in nociception and chemotaxis. By patch-clamp recordings from cultured *C. elegans* L1 muscle cells, we described for the first time the properties of BE-elicited single-channel and macroscopic currents. Our study provides novel information aiming at the elucidation of the molecular function and pharmacology of the nAChR family. It also contributes to the understanding of the molecular basis of anthelmintic action, which paves the way for the development of novel drugs.

1001V **Major sperm proteins expressed in ADL chemosensory neurons require the NRDE-3 somatic nuclear RNAi pathway** Maria C. Ow[1], Abdul Rouf Dar[2], Rebecca A. Butcher[2], Sarah E. Hall[1,11]Syracuse University, [2]University of Florida

Environmental conditions experienced early in the life of an animal can result in gene expression changes during adulthood. We have previously shown that *C. elegans* animals that experienced the developmentally arrested and stress resistant dauer stage (postdauers) retain a cellular memory of early-life stress that manifests during adulthood as genome-wide changes in gene expression, chromatin states, and altered life history traits. One consequence of developmental reprogramming in *C. elegans* postdauer adults is the downregulation of expression of a TRPV channel gene, *osm-9*, in the ADL chemosensory neurons which results in the reduced avoidance to a pheromone component, ascr#3. This reduction in ascr#3 avoidance requires the somatic nuclear RNAi pathway.

To investigate the role of the somatic nuclear RNAi pathway in regulating the developmental reprogramming of *osm-9* in ADL due to early-life stress, we profiled the mRNA transcriptome of control and postdauer ADL in wild-type and *nrde-3* mutant adults. We find that the wild-type ADL transcriptome expresses germline-expressed genes. NRDE-3, the effector of the somatic nuclear RNAi pathway, plays a critical role in regulating the expression of germline-expressed genes in ADL neurons, such as major sperm proteins (MSPs) genes, even under non-stressful growth conditions. Loss of MSPs function, through mutation of the *gsp-3* and *gsp-4* sperm-specific PP1 phosphatases, results in the abrogation of ascr#3 avoidance and aberrant olfactory behavior. We also show that an Argonaute pseudogene, *y49f6a.1* (*wago-11*), is expressed in ADL and is required for ADL chemosensory function. Overall, our results suggest that small RNAs and reproductive genes program the ADL mRNA transcriptome during their developmental history and highlight a nexus between neuronal and reproductive networks in calibrating animal neuroplasticity.

1002V **ALK/SCD-2-dependent expression of DAF-7 from the ASJ neurons couples bacterial food ingestion to foraging state dynamics in *C. elegans*** Sonia Boor[1,2], Joshua Meisel[3], Dennis Kim[11]Boston Children's Hospital, [2]Biology, MIT, [3]MGH

Animal internal state is modulated by nutrient intake, resulting in behavioral responses to changing food conditions. Whereas neuronal circuitry responsive to changes in food availability has been increasingly characterized, less is known about the role for changes in neuronal gene expression in shaping internal state. *C. elegans* exhibits a food-dependent two-state foraging behavior that alternates between exploration and exploitation behavioral states known as roaming and dwelling. Here, we identified a role for the *C. elegans* ortholog of Anaplastic Lymphoma Kinase (ALK), SCD-2, in the regulation of a neuroendocrine gene expression loop that couples the ingestion of bacterial food to the dynamics of foraging behavior. We showed that ALK/SCD-2 controls the expression of the neuronal TGF-beta, DAF-7, in the ASJ chemosensory neurons, which we found to be inhibited by the ingestion of bacterial food. In turn, we determined that DAF-7 expression from the ASJ neurons promotes roaming state behavior by extending the duration of the roaming period. Our data reveal a pivotal role for ALK/SCD-2 regulation of food-dependent, dynamic DAF-7 expression that functions in a physiological positive-feedback loop that facilitates behavioral state changes in response to changing food conditions.

1003V **Neuronal FMRFamide neuropeptide signaling controls the activation of the head mesodermal cell (hmc) during a rhythmic behavior in *C. elegans*** Mingxi Hu, Ukjin Choi, Derek SieburthZilkha Neurogenetic Institute

FMRFamides are an evolutionarily conserved family of neuropeptides that are highly expressed in the nervous system and play important roles in behavior, energy balance and reproduction. Here, we show that FMRFamide signaling is critical for the anterior body wall muscle contraction (aBoc) step of the defecation motor program (DMP), and functions by controlling the generation of calcium responses in a single cell of previously unknown function, hmc. FLP-22 is released from a bifunctional motor neuron AVL in response to pacemaker signaling and activates the G protein coupled receptor (GPCR), FRPR-17, in hmc. FRPR-17 activates a G alpha s-protein kinase A (PKA) signaling cascade in hmc, leading to the generation of a single large calcium transient in hmc every 50 seconds that occurs in phase with AVL activation and aBoc. Genetic ablation of hmc results in missing aBoc steps during the DMP. Similarly, *flp-22* or *frpr-17* mutations leads to missing aBocs and to the near absence of calcium transients in hmc, and expression of FRPR-17 selectively in hmc restores normal aBoc and hmc calcium transient frequency in *frpr-17* mutants. hmc itself is not contractile but is functionally coupled to neck muscles through gap junctions composed of UNC-9/innexin. aBoc and hmc activation are inhibited by signaling from a second FMRFamide-like neuropeptide, FLP-9, which functions through its GPCR, FRPR-21, in hmc. Overexpressing FLP-9 eliminates aBoc and hmc activation, whereas *flp-9* or *frpr-21* mutations restore aBoc

and hmc activation to animals lacking FLP-22/FRPR-17 signaling. This study reveals a new function for FMRFamides signaling in controlling a rhythmic behavior, identifies opposing FMRFamides in shaping the activation of a target cell through volume transmission, and establishes a function for hmc in the aBoc circuit.

1004V **Neural circuits of oxygen, carbon dioxide, and temperature that generate cold acclimation diversity** Misaki Okahata[1], Akane Ohta[1,2], Sawako Yoshina[3], Yohei Minakuchi[4], Toru Miura[1], Shohei Mitani[3], Atsushi Toyoda[4], Atsushi Kuhara[11]Graduate school of Natural Science & Institute for Integrative Neurobiology, Konan University, [2]Konan University, [3]Tokyo Women's Medical University, [4]National Institute of Genetics

Temperature is one of the most important ambient factors. To elucidate the mechanism in diversity of temperature response of animal, we are studying natural variation of cold acclimation in C. elegans natural variation. AB1 from Australia can rapidly acclimate to new temperature, in contrast, CB4856 from Hawaii can acclimate slowly (Okahata et al., *JCPB*, 2016). We identified *VH* gene as a responsible gene for the natural variations of cold acclimation, by using NGS and SNP analysis. When animals were cultivated at 25 degrees, *VH*mutant showed abnormal cold acclimation and abnormal development. *VH* gene was expressed in BAG sensory neurons, which receive oxygen and carbon dioxide. To explore the downstream molecule of *VH* gene, we performed RNA sequencing analysis. *VH* mutant showed the decrement of expression level in *sax-2* and *crml-1* genes involved in axon guidance, and abnormal axon branching in BAG neuron. We previously reported that ADL thermo-responsivity and cold acclimation are affected by oxygen sensory signaling from upstream URX oxygen sensory neuron. (Okahata et al., *Science Advances*, 2019). We therefore hypothesized that oxygen and carbon dioxide signaling from the BAG also modulates cold acclimation by affecting the ADL thermo-responsivity via *VH* in BAG. *VH* mutant exhibited abnormal decrement of ADL thermo-responsivity. Besides, the mutants defective in oxygen receptor, GCY-33 and putative carbon dioxide receptor, GCY-9 showed abnormal cold acclimation and ADL thermo-responsivity, suggesting that oxygen and carbon dioxide sensory-information from BAG also affect ADL temperature response. We then considered whether oxygen and carbon dioxide signaling generates the diversity of temperature response. In 20% oxygen concentration condition, AB1 showed significant increment of cold acclimation and ADL thermo-responsivity compared with CB4856. Whereas, both AB1 and CB4856 showed similar cold acclimation and ADL thermo-responsivity in 5% oxygen condition. Although cold acclimation and ADL thermo responsivity of CB4856 were almost not affected by carbon dioxide concentration, the cold acclimation and ADL thermo responsivity of AB1 were altered by carbon dioxide concentration. These suggested that ambient oxygen and carbon dioxide information from BAG would modulate ADL thermo-responsivity, resulting in the diversity of cold acclimation.

1005V **Lipid metabolism-related genes involved in heat tolerance as revealed by transcriptome analysis of EMB-4** Akane Ohta[1,2,3], Yuki Sato[1], Kazuho Isono[4], Takuma Kajino[4], Teruaki Taji[4], Atsushi Kuhara[5,61]Biology, Grad. School of Konan University, [2]Biology, Konan University, [3]Institute for Integrative Neurobiology Konan University, [4]Bioscience, Tokyo University of Agriculture, [5]Institute for Integrative Neurobiology, Grad. School of Konan University, [6]AMED / PRIME

Adapting to changing temperatures is essential for either plants or animals to survive and proliferate on the earth. We found that cold and heat tolerance in the nematode *Caenorhabditis elegans* are regulated in an opposite way by using EMB-4 splicing factor. *emb-4* is a homologous gene of heat-tolerance responsible gene of Arabidopsis thaliana, and these are ortholog of human RNA-binding protein Aquarius (AQR). Mutation of *emb-4* gene increased the survival of cold tolerance, however, decreased heat tolerance, suggesting that EMB-4 regulates cold and heat tolerance in a negative and positive direction, respectively. Previous reports indicated that EMB-4 binds to RNAs for regulating various gene expressions in the germline of *C. elegans*. To investigate genes whose expression was affected by loss of *emb-4* upon thermal stimuli, we conducted transcriptome analyses of *emb-4* mutants with temperature changes. Worms were collected and subjected to RNA-seq analysis after cultivation of four conditions; (1) 20°C-cultivation, (2) 20°C-cultivation then 2°C-incubation for 9 hours, (3) 20°C-cultivation then 32°C-incubation for 1 hours, and (4) 20°C-cultivation then 32°C-incubation for 13 hours. In these conditions, we compared RNA levels of *emb-4* mutant to wild-type. We found that the numbers of upregulated genes were (1) 1103, (2) 799, (3) 1321, (4) 480, and that the numbers of down-regulated genes were (1) 173, (2) 662, (3) 133, (4) 161 of each collection. Gene ontology analysis revealed that EMB-4 was involved in regulating the expression levels of genes that are involved in stress response, metabolism, extracellular material, and non-coding RNA. Those genes may include temperature tolerance-related genes. Therefore, we measured cold and heat tolerance of approximately 20 mutants whose expression was significantly altered by *emb-4* mutation. In particular, a phospholipid scramblase and acid sphingomyelinase, which are involved in lipid metabolism, were involved in heat tolerance. These data imply that lipid metabolism or other stress responses are involved in temperature tolerance, in part downstream of EMB-4.

1006V **Long and short isoforms of the UNC-6/Netrin receptor UNC-5 have distinct roles in VD/DD axon guidance and VD growth cone protrusions in *C. elegans*** Snehal Mahadik, Erik Lundquist Molecular Biosciences, University of Kansas

UNC-6/Netrin and its receptors UNC-40/DCC and UNC-5 regulate dorsal VD growth cone outgrowth by regulating growth cone

polarity and subsequent protrusion of growth cone lamellipodia and filopodia in response to this polarity (the Polarity/Protrusion model). In response to UNC-6/Netrin, UNC-40 stimulates protrusion dorsally, and UNC-5 inhibits protrusion ventrally, resulting in dorsally-directed growth cone migration away from UNC-6/Netrin. *unc-5* encodes full-length long isoforms, as well as a short isoform truncated at the C-terminus and lacking most cytoplasmic domains (*unc-5B)*. *unc-5* null mutant VD growth cones (lacking both long and short isoforms) display unpolarized and excessive protrusion, whereas activated *myr::unc-5* results in small growth cones with reduced protrusion. Long-isoform-specific mutants also display unpolarized and over-protrusive growth cones, but are less uncoordinated and have less severe axon guidance defects than nulls. This suggests that long-isoform-specific mutants are not null and that the *unc-5* short isoform contributes to axon guidance. Precise genomic deletion of *unc-5B* resulted in unpolarized growth cones with reduced protrusion. It also affects the dorsal asymmetrical accumulation of F-actin but not MT+ end accumulation. This suggest that in addition to differentially regulating growth cone morphology, UNC-5B plays a role in regulating cytoskeletal dynamics to maintain dorsal polarity of filopodial protrusions. Transgenic expression of *unc-5A* long isoform rescued uncoordinated locomotion, axon guidance defects, and growth cone protrusion of *unc-5*null mutants. Transgenic expression of *unc-5B* short rescued uncoordinated locomotion and axon guidance defects, but did not rescue excess protrusion of *unc-5* null mutants. In sum, these results show that the long and short isoforms of UNC-5 play differential roles in growth cone migration. The long isoform UNC-5A inhibits growth cone protrusions and the UNC-5B short isoform is pro-protrusive in nature and the balance between the two isoforms is crucial for the development of VD/DD neurons.

1007V **Neural modulation of behavioral state transitions in foraging strategies in *C. elegans*** Maria Gabriela Blanco[1], Jeremy Florman[2], Mark Alkema[3], María José De Rosa[1], Diego Rayes[11]Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Instituto de Investigaciones Bioquímicas de Bahía Blanca, [2]Departmen of Neurobiology, University of Massachusetts Chan Medical School, [3]Department of Neurobiology, University of Massachusetts Chan Medical School

Adequate feeding behavior is essential for animal survival and it is regulated not only by the digestive system but also by the nervous system (NS). The NS allows the animal to respond flexibly to changes in the environment depending on the availability of food and the nutritional internal state. Despite feeding behaviors have been studied for decades, understanding the mechanisms involved in different animals´ responses to food depending on its internal state (satiated or fasted/stressed) is still a major challenge. Referred to as the “happiness hormone”, serotonin (5-HT) has been shown to increase with food stimulus and modulate feeding in different animals, suggesting that the role of 5-HT is conserved in nature. On the other hand, noradrenaline (NA), implicated in triggering a stress response, is involved in appetite control by reducing food ingestion. Interestingly, there are reports showing that a lesion of the serotonergic system enhances the effect of noradrenergic drugs. These findings indicate an interaction between serotonergic and noradrenergic signaling. However, the mechanism and relevance of this interplay are not entirely clear. Therefore, our goal is to investigate the molecular processes underlying this interaction.

The complexity of the mammalian brain complicates the study of neuronal processes. The nematode *Caenorhabditis elegans* is suitable for understanding neuronal signaling because of its simple and well-described nervous system. We found that during prolonged fasting, animals decrease their locomotion, which can be resumed by adding tyramine (TA), the analog of NA in invertebrates. 5-HT produces the opposite effect by reducing locomotion, suggesting that 5-HT acts antagonistically to TA. Moreover, it has been shown that when the environment improves and fasted animals encounter food, they release 5-HT to slow their locomotion and promote feeding. Interestingly, we found that this slowing response and the activity of the serotonergic neurons upon food encounter are enhanced in TA-deficient mutants compared to wild-type animals. Given that we also show that TA levels decrease during fasting, we hypothesize that this disinhibits the serotonergic neurons and favors their activity upon refeeding, allowing the animal to exploit the new source of food. Considering the conservation of neuronal components, we believe that our results may contribute to the understanding of the nervous control of state dependent foraging strategies.

1008V **Temperature regulates glia morphogenesis through thermosensory circuits** Junyu Zheng[1], Shaochen Wang[1], Mengqing Wang[1], Zhiyong Shao[21]Fudan University, [2]Institutes of Brain Science, Fudan University

Astrocytes are the most abundant macroglia in the brain and play critical roles in regulating neural development and functions. The diversity of astrocyte functions is largely determined by the heterogeneity of its morphology. However, how the astrocyte morphology is established remains largely unknown. Temperature perturbation affects neuronal development and function in both vertebrates and invertebrates, and high temperature has been shown to suppress astrocyte division and viability in tissue culture. However, if and how the temperature affects astrocyte morphogenesis *in vivo* remain unknown. In this study, we found that high cultivate temperature (26℃) promoted *C. elegans* astrocyte CEPsh endfoot extension, which requires thermosensory AWC neurons and the postsynaptic AIY interneurons. We further demonstrated that two glutamate gated chloride channels, GLC-3 and GLC-4, were required for the glia extension. Finally, we identified that the guanyl-nucleotide exchange factor EPHX-1 and GTPase CDC-42 act cell-automatically in the glia to regulate endfoot extension under high temperature. Collectively, these data suggest a model that high temperature acts through a thermosensory circuit to modulate the glia morphogenesis in *C. elegans*, which provides a novel mechanism underlying glia morphogenesis and may provide insights into high-temperature related

developmental disorders.

1009V **How does *C. elegans* recognize the bacterial odors of its microbiome?** Tiam Farajzadeh[1], Victor Z Chai[2], Yufei Meng[2], Charles J Taylor[1], Elizabeth E Glater[21]Chemistry, Pomona College, [2]Neuroscience, Pomona College

Chemosensation plays a central role in driving behavioral outcomes, including selecting food sources, finding mates, and avoiding threats and predators. How the nervous system discriminates among natural chemosensory stimuli, which are often comprised of complex blends of molecules, is not well understood. The nematode *C. elegans* is an excellent model organism to address this question. While much is known about the neurons and signaling pathways involved in responses of *C. elegans* to individual chemicals (reviewed in Ferkey et al., 2021), the mechanisms underlying detection of complex natural stimuli remain poorly understood. We are investigating how *C. elegans* discriminates among different odor blends released by bacteria, their major food source. Specifically, we are examining CeMBio a simplified microbiome consisting of 12 species representative of the bacteria in *C. elegans* natural environment (Dirksen et al., 2020). We found that *C. elegans* showed a strong preference for the odor of three of the ten tested bacterial isolates over its standard laboratory food source, *E. coli* OP50. We are using gas chromatography coupled with mass spectrometry (GC-MS) to identify the volatile chemicals released by the bacterial isolates. Our goal is to define the chemical signatures of the *C. elegans* microbiome and the neuronal circuitry involved in discriminating among these naturally occurring odor mixtures.

1010V **Investigation of the molecular mechanisms underlying the neuronal preconditioning response to anoxia in *C. elegans*** Ginger Watzinger, Heather L Bennett Biology, Trinity College

Neurons require oxygen for proper function and insufficient oxygen (hypoxia), or the complete lack of oxygen (anoxia) causes neuronal dysfunction and death. Sub lethal stress prior to a hypoxic or anoxic insult “preconditioning” enhances survival and can protect neurons from subsequent hypoxic or anoxic insult. The cellular mechanisms and neural circuitry underlying the preconditioning response to oxygen deprivation has not been fully characterized.

We are using *Caenorhabditis elegans* to investigate the biological basis underlying the neuronal preconditioning response. *C. elegans* can be preconditioned to anoxia and we have previously shown that hyperpolarization of cholinergic, GABAergic, or body wall muscle activity prior to a 48-hour anoxic insult increases survival to anoxia in larval stage 4 (L4) animals (Bennett *et al.,* Genes Brain Behavior 2020). We hypothesized that neuronal preconditioning was dependent on known stress genes or stress response pathways that have been implicated in the hypoxia response. We tested animals that expressed the histamine gated chloride channel in cholinergic neurons in animals that also had a loss of function mutation in a stress gene in our anoxia survival assay. We find that hyperpolarization of cholinergic neurons prior to 48-hours of anoxia increased survival, which is consistent with published results. However, loss in the ceramide synthase gene, *hyl-2,* suppressed the increase survival in cholinergic preconditioned animals.

Ongoing studies aim to elucidate the molecular mechanisms by which *hyl-2* regulates the preconditioning response to anoxia, as well as identify the specific neurons where *hyl-2* functions to regulate anoxia survival.

1011V **Bioeffects of therapeutic ultrasound in the motor and sensory nervous systems of *C. elegans*** Louise Steele, Brandon Krall, Vivian Conrad, Paige DoremusKent State University

Our lab uses *C. elegans* to study the biological effects of ultrasound. After exposure to therapeutic ultrasound, adult hermaphrodites had dose-dependent reductions in several parameters, including mobility (Steele *et al.*, 2021). Rather than thrashing rapidly in the typical sinusoidal pattern, some worms exhibited a slow, irregular movement that we called “writhing”. In the current study, we documented that many writhing worms recovered relatively quickly on agar. To explain the reversibility of this effect, we hypothesized that ultrasound alters neurotransmission in the worms’ motor nervous system. Writhing worms were hypersensitive to aldicarb, which suggested that acetylcholine signaling was indeed affected. To rule out the possibility that writhing worms were simply more permeable to aldicarb, we stained them with a fluorescent dye that only enters cells with compromised plasma membranes. Based on those results, at least some writhing worms were unlikely to be taking up aldicarb more readily than unexposed shams were. Further work will determine if the defect in neurotransmission is pre- or post-synaptic. In addition, we hypothesized that ultrasound may affect the worms’ sensory nervous system. We exposed L1 larvae to a half-lethal dose of ultrasound, let them grow to adulthood, and tested them in chemotaxis assays. Despite moving well on agar, their chemotaxis index was reduced compared to that of unexposed shams. Thus, ultrasound exposure appeared to impair the worms’ ability to sense a chemoattractant, and the impairment seemed to persist into adulthood. Further work will help to identify the change(s) responsible. *C. elegans* is a valuable model for understanding ultrasound’s effects at the behavioral, cellular, and molecular levels.

1012V **Roles of a non-canonical hedgehog-like pathway in the assembly of the *C. elegans* nerve ring** Francesca Caroti[1],

Chiara Mungo[1], Georgia Rapti[2,3,41]EMBL, [2]Developmental biology, EMBL, [3]Epigenetics and Neurobiology Unit, EMBL, [4]Interdisciplinary Center of Neurosciences, EMBL

Neural circuits consist of large cell numbers of neuronal and glial cell types. The complexity of their functional architecture relies on complex cellular and molecular interactions of their components. These interactions are challenging to study *in vivo* due to the in-accessibility of embryonic stages for imaging and genetic manipulations, in most systems. The *C. elegans* brain-circuit, "nerve ring", offers a powerful setting to study these underpinnings, due to its stereotyped anatomy, accessible embryos, easy imaging and genetic manipulations in single-cell resolution. We study the molecular events of assembly, using advanced genetic, genomics, live embryonic imaging, and functional studies.

We previously demonstrated that the nerve ring, consisting of 183 axons and 4 astrocyte-like glia, forms in a hierarchical manner. Specific pioneer neurons and glia initiate assembly and guide follower components in stereotypical spatiotemporal patterns. We characterized pioneer-cell-derived molecular cues driving assembly and revealed an array of molecular synergies ensuring its fidelity. Such synergies can hinder identification of new cues and had plagued genetic analysis of circuit formation in the past.

To tackle synergies and identify hidden factors cooperating for circuit assembly, we developed specific modifier genetic screens. We combine these with mutagenesis, candidate approaches, and sensitized backgrounds including a previously-identified Chimaerin mutant with mild axon defects. We isolated a new mutant enhancer that causes defects in follower axon navigation. Using mapping and rescue experiments we identified the causal mutation in the gene *grl-24*. GRL-24 is a secreted cue of a non-canonical Hedgehog-like pathway of *C. elegans* that remains largely understudied.

Our genetic analysis and genomic CRISPR approaches suggest that the isolated grl-24 mutation causes a dominant effect. We demonstrate that GRL-24 is needed specifically for navigation of follower neurons, without affecting pioneer neurons. Furthermore, through additional genetic screens, we identify specific contributions of other genes of the Hedgehog-like pathway in follower axon navigation. We are also characterizing the spatiotemporal roles and primary mechanistic functions of GRL-24 in different cell types and developmental stages. We will report our results in these directions. Our work uncovers that the non-canonical Hedgehog-like pathway of *C. elegans* has key hidden roles in brain assembly.

1013V **Understanding the role of gene expression regulation in nicotine induced neuroprotection** Hagit Cohen Ben-Ami[1], Millet Treinin[21]Hebrew University, Hadassah Medical school, [2]Medical Neurobiolgoy, Hebrew University - Hadassah Medical School

Tobacco smoking reduces the risk for Parkinson's disease while increasing the risk for several other diseases. This effect is likely mediated by tobacco derived nicotine activating nicotinic acetylcholine receptors (nAChRs). Previously we have established a *C. elegans* model for nicotine induced neuroprotection of dopaminergic neurons (Nourse et al., iScience 2021). Using this model we have shown that chronic nicotine exposure reduces 6-OHDA induced degeneration of dopaminergic neurons via a pathway involving nAChRs and calcium influx into mitochondria.

Further analysis of nicotine's neuroprotective effects demonstrated that protective effects of pre-exposure and acute exposure to nicotine are additive. This suggests separable pathways enabling nicotine-induced neuroprotection. Moreover, the prolonged protection afforded by pre-exposure to nicotine suggests involvement of gene expression regulation in nicotine induced neuroprotection. Indeed, chronic nicotine exposure upregulates expression of genes involved in the mitochondrial stress response. However, this upregulation is seen late in development may be a byproduct of mitochondrial stress induced by calcium influx into mitochondria.

To better understand the mechanisms enabling nicotine induced neuroprotection we are analyzing the effects of nicotine on gene expression in neurons. We believe that this analysis will identify genes whose regulation by nicotine contributes to nicotine induced neuroprotection.

1014V **Single-cell RNA-seq analysis reveals extensive sexual dimorphism of the sex-shared PLM neuron** Hagar Setty[1], Rizwanul Haque[2], Gil Stelzer[3], Ramiro Lorenzo[4], Yehuda Salzberg[2], Patrick Laurent[5], Meital Oren-Suissa[21]Weizmann Institute of Science, [2]Brain Sciences, Weizmann Institute of Science, [3]Department of Life Sciences and Core facilities, Weizmann Institute of Science, [4]Centro de Investigacion Veterinaria de Tandil (CIVETAN), Universidad Nacional del Centro (FCV-UNCPBA), Tandil, Argentina, [5]. Laboratory of Neurophysiology, UNI, Universite´ Libre de Bruxelles

Sexually reproducing animals display sex-specific behaviors wired onto dimorphic connectivity patterns in the nervous system. Although sexual dimorphism in the nervous system is extensively studied, little is known about the molecular mechanisms that underlie the development of sexually dimorphic neuronal circuits.

Single cell RNA-sequencing of the entire nervous system of hermaphrodites and males recently generated in our lab (Haque et al, unpublished results) provides a unique opportunity to explore sexually dimorphic properties in the nervous system and enables to investigate the molecular landscape of sex-shared neurons.

Using computational analysis, we filtered the single-cell clusters of sex-shared neurons according to the number of differentially expressed genes (DEGs) between the sexes. This analysis revealed 365 DEGs in the sensory neuron PLM, the highest number of DEGs among the clusters of sensory neurons examined.

To test whether the sexually dimorphic transcriptional profile of PLM translates into behavior, we examined gentle touch mechanosensation behavior, which is known to be mediated by PLM, in both sexes. We discovered that the response of the males to gentle touch is higher compared to the response of the hermaphrodites, suggesting a functional, previously uncharacterized, dimorphic role for PLM. We further present molecular candidates that are expressed differently between the sexes in PLM and could contribute to this behavior. Additional analysis discovered "molecular bias" in PLM, showing a larger number of differentially expressed genes towards the male direction compared to hermaphrodite, suggesting a more prominent role for PLM in males, and correlating with our behavioral analysis.

Moreover, combining the CeNGEN data with our transcriptomic data suggests that the gene F10A3.11 is expressed solely in PLM. In future plans, we plan to activate PLM optogenetically using the promoter of this gene and observe the response of the worms in both sexes.

Overall, this study will contribute to the understanding of how sexually dimorphic molecular mechanisms lead to sexually dimorphic behaviors.

1015V **Using a TurboID-based approach to investigate how memories form in *C. elegans*** Aelon Rahmani, Yee Lian Chew
Flinders University

Learning enables animals to take advantage of previous experiences so to choose behaviours that ensure survival. Previous studies have reported that memory involves changes in the nervous system proteome, including the expression and/or localisation of proteins that regulate neuronal communication, but many of the molecular pathways reported to affect memory remain partially defined. In this poster, I will describe my progress toward performing an unbiased screen of the nervous system proteome during memory formation, to potentially reveal novel mechanisms within this biological process. Specifically, I will use the protein-labelling method TurboID in the nematode *C. elegans* while they encode memory of salt aversive learning. This form of learning involves classical conditioning with a normally appetitive cue (sodium chloride/salt) and a physiologically stressful stimulus (starvation). TurboID can be used in many animals including *C. elegans*, and it involves the use of a biotin ligase enzyme TurboID/miniTurbo that tags nearby proteins with the chemical biotin, so that these labelled proteins can be pulled-down for identification by mass spectrometry. These enzymes are not naturally made in the worm; I have currently performed (1) behavioural assays confirming that transgenic *C. elegans* that express a miniTurbo enzyme can learn normally, and (2) western blot assays to validate that miniTurbo can biotinylate proteins while these transgenic animals learn. Overall, the findings described in this poster will provide a basis to use TurboID in the worm to explore the molecular mechanisms underlying learning.

1016V **Sexually dimorphic architecture and function of a mechanosensory circuit in *C. elegans*** Hagar Setty[1], Yehuda Salzberg[1], Shadi Karimi[2], Michael Krieg[2], Meital Oren-Suissa[1]1Brain Sciences, Weizmann Institute of Science, [2]ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology

How sensory perception is processed by the two sexes of an organism is still only partially understood. Despite some evidence for sexual dimorphism in auditory and olfactory perception, whether touch is sensed in a dimorphic manner has not been addressed. Here we find that the neuronal circuit for tail mechanosensation in *C. elegans* is wired differently in the two sexes and employs a different combination of sex-shared sensory neurons and interneurons in each sex. Reverse genetic screens uncovered cell- and sex-specific functions of the alpha-tubulin *mec-12* and the sodium channel *tmc-1* in sensory neurons, and of the glutamate receptors *nmr-1* and *glr-1* in interneurons, revealing the underlying molecular mechanisms that mediate tail mechanosensation. Moreover, we show that only in males, the sex-shared interneuron AVG is strongly activated by tail mechanical stimulation, and accordingly is crucial for their behavioral response. Importantly, sex reversal experiments demonstrate that the sexual identity of AVG determines both the behavioral output of the mechanosensory response and the molecular pathways controlling it. Our results present extensive sexual dimorphism in a mechanosensory circuit at both the cellular and molecular levels.

1017V **Body Stiffness Is A Mechanical Cue to Facilitate Contact-mediated Mate Recognition in *C. elegans*** Jen-Wei Weng[1], Heenam Park[2], Claire Valotteau[3], Rui-Tsung Chen[1], Nathalie Pujol[4], Paul W. Sternberg[2], Chun-Hao Chen[1]1Institute of Molecular and Cellular Biology, College of Life Science, National Taiwan University, [2]Division of Biology and Biological Engineering, California Institute of Technology, [3]Aix-Marseille Univ, INSERM, CNRS, LAI, Turing Centre for Living Systems, [4]Aix Marseille Univ,

INSERM, CNRS, CIML, Turing Centre for Living Systems

Physical contact is prevalent in the animal kingdom to recognize suitable mates by decoding information about sex, species, and maturity. Although chemical cues for mate recognition have been extensively studied, the role of mechanical cues remains elusive. Here we show that *C. elegans* males recognize conspecific and reproductive mates through short-range cues, and the attractiveness of potential mates depends on the sex and developmental stages of the hypodermis. We found that a particular group of cuticular collagens is required and sufficient for mate attractiveness. These collagens maintain body stiffness to sustain mate attractiveness but do not affect the surface property that evokes the initial step of mate recognition, suggesting that males utilize parallel sensory mechanisms to recognize suitable mates. Manipulations of body stiffness via physical interventions, chemical treatments, and 3D-printed bionic worms indicate that proper body stiffness is a mechanical cue for mate recognition and increases mating efficiency. Our study thus extends the repertoire of sensory cues of mate recognition in *C. elegans* and provides a paradigm to study the vital role of mechanosensory cues in social behaviors.

1018V **Real-time dopamine binding during appetitive butanone forgetting assay in *Caenorhabditis elegans*** Anna McMillen, Yee Lian Chew Flinders University

Dopamine is a neurotransmitter that plays an important role in learning, memory, and forgetting processes. *C. elegans* has had a significant history as a model for learning and memory studies with dopamine but the role of dopamine in the forgetting process is not as fully characterized. Although there has been some work using invertebrate models to investigate the mechanisms behind the role of dopamine in forgetting they are currently limited and predominately occur in *D. melanogaster*. The role of dopamine in forgetting using an appetitive butanone learning assay is examined in the worms. Molecular tools have been used to begin mapping neural circuits pertaining to learning and memory in *C. elegans*. Although these molecular tools have illuminated important genes and mechanisms so far, the real-time visualization of these mechanisms has yet to be fully explored due to limitations in the tools. The dopamine sensor GRAB-DA was developed and has been shown to successfully bind dopamine in models such as mice and human cells but has yet to be validated for use in *C. elegans*. Multiple versions of this sensor are examined for use in the worms. We hope to use the GRAB-DA sensor in *C. elegans* during an appetitive butanone learning assay to examine the real-time binding of dopamine during the forgetting process.

1019V **Intrinsic Sex and Vulval Cues Shape Sexually Dimorphic Branching and Functions of PVP interneurons in *C. elegans*** Jia-Bin Yang, Rui-Tsung Chen, Chun-Hao ChenInstitute of Molecular and Cellular Biology, College of Life Science, National Taiwan University.

Intrinsic sexual states or non-autonomous signals have been shown to shape the sexual dimorphism of the nervous system, whereas whether these factors orchestrate to sculpt sexually dimorphic morphology remains unknown. Here, we find that the sex-shared PVP cholinergic interneurons exhibit sexually dimorphic connectivity and morphology for sexually dimorphic behaviors. Specifically, PVP neurons develop sexually dimorphic collateral branches in hermaphrodites to modulate egg-laying behaviors. The development of sexually dimorphic branching begins at the time of sexual maturation with the enrichment of F-actin, and the mature PVP branches accommodate IDA-1-positive vesicles, suggesting the role of collateral branches in synaptic transmission. Interestingly, we show that nutritional status regulates branch morphology with wing-like or rod-like shapes at the branch terminal. Genetic analysis demonstrates that branch formation requires intrinsic factors consisting of the sexual state and the FOXO/DAF-16 transcription factor, and external vulva cues from VulE and VulF. We show that these intrinsic and external signals converge on the control of F-actin enrichments for branch formation. Our study thus provides mechanistic insight into how intrinsic sexual states and external signals coordinate to shape sexually dimorphic connectivity.

1020V **Neural Basis of A Sensory-evoked Behavioral State in *C. elegans* Males** Kai-An You, Yin-Chen Lin, Tse-Yu Chen, Chun-Hao Chen Institute of Molecular and Cellular Biology, College of Life Science, National Taiwan University

Animals integrate internal needs and environmental cues to display behavioral states that offer flexible, scalable, and persistent actions for adaptations. Previous studies indicate that *C. elegans* males show a long-term reduction of locomotion coverage upon contacting suitable mates, while the circuit mechanism remains unknown. Here, we find that brief contact with suitable mates in able to evoke a persistent behavioral state that abruptly restricts locomotion coverage by increasing reversals for minutes. The local search state requires physical contact by male tails but not pheromones, suggesting that tail sensilla are needed. Indeed, transient optogenetic activation of ray type B neurons (RnBs), a group of sensory neurons for contact response in males, recapitulates persistent behavioral changes associated with the recurrent activity. In addition, we show that glutamatergic LUA interneurons and a CRF-like GPCR receptor SEB-3 are necessary to sustain the local search state evoked by RnBs activation. Genetic analyses indicate that LUAs likely distribute glutamate signals through multiple glutamatergic receptors on commanding AVA interneurons for repeated reversals. Our study thus uncovers a glutamatergic circuit underlying a local search state of males upon contacting suitable mates and provides molecular insights into neural mechanisms of sensory-evoked behavioral states.

1021V **The 3'UTR of *kpc-1*/furin promotes dendritic mRNA transport and local protein synthesis to regulate dendrite branching and self-avoidance of a nociceptive neuron** Mushaine Shih[1], Yan Zou[2], Tarsis Ferreira[3], Nobuko Suzuki[1], Kelsie Eichel[4], Chiou-Fen Chuang[1], Chieh Chang[11]University of Illinois at Chicago, [2]School of Life Science and Technology, ShanghaiTech University, [3]Cincinnati Children's Hospital Research Foundation, [4]Stanford University

A recently reported Schizophrenia-associated genetic variant in the 3'UTR of the human furin gene, a *kpc-1* homolog, highlights the important role of its 3'UTR in neuronal development (Schrode, et al., 2019 *Nature Genetics*). We isolate three *kpc-1* mutants that display dendrite branching and self-avoidance defects in PVD neurons and defective male mating behaviors. We show that the *kpc-1* 3'UTR is required for dendrite branching and self-avoidance. The *kpc-1* 3'UTR facilitates mRNA localization to branching points and contact points between sibling dendrites and promotes local protein synthesis. We identify a secondary structural motif in the *kpc-1* 3'UTR required for dendrite self-avoidance. Animals with *dma-1* receptor over-expression exhibit similar dendrite branching and self-avoidance defects that are suppressed with *kpc-1* over-expression. Our results support a model where 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 mechanosensory neurons required for male courtship behaviors.

1022V **Chemosensory integration of food availability and crowdedness during developmental decision-making relies on insulin-like neuropeptide signaling** Mark G Zhang[1], Seyedehmaedeh Seyedolmohadesin[2], Vivek Venkatachalam[2], Paul W Sternberg[31]Biology, California Institute of Technology, [2]Northeastern University, [3]California Institute of Technology

A fundamental question regarding the *C. elegans* dauer decisions that has remained unanswered is how animals integrate conflicting sensory cues such as food availability and crowdedness (conveyed by pheromone). Previous reports have implicated the ASJ chemosensory neuron and neuropeptides such as *ins-6* in the dauer exit decision. To validate these finding, we developed an ethologically relevant dauer exit assay using wild-type pheromone-induced dauers and confirm that neuropeptides collectively and *ins-6* specifically promotes dauer exit. We then sought to understand whether and how *ins-6* signaling in ASJ mediates the sensory integration process. Using fluorescent reporter assays, we find that in the first three hours of dauer exit, *ins-6* is transcriptionally upregulated in ASJ, INS-6 is secreted throughout the body cavity, and *ins-6* transcription responds strongly to pheromone decrease and responds modestly to food increase but only at intermediate pheromone concentrations. To examine how neuronal activity of ASJ and other sensory neurons relates to food, pheromone, or a combination of the two, we performed calcium imaging in ciliated sensory neurons of L4 larvae. Surprisingly, we find that ASJ responds to food but not pheromone, even though *ins-6* in ASJ reflects primarily pheromone levels. We also find that knocking out *unc-31* completely abrogates the *ins-6* signal, while knocking out *unc-13* has little to no effect. We therefore postulate that ASJ receives neuropeptidergic input from other pheromone-sensing neurons to calculate an internal metric of food and pheromone concentrations. In sum, we show how insulin-like peptides regulate the dauer exit developmental decision by acting as environmentally responsive signaling molecules secreted by chemosensory neurons and targeted to downstream target tissues.

1023V **Elucidating the sensory mechanism and ecological relevance of a *C. elegans* maternal egg-laying behavior in 3D environments** Eunha Chang, Tong Young Lee, Jin I Lee Yonsei University

Animals' behaviors are adapted to specific environments to increase their evolutionary success. This is particularly true for parental behaviors that directly affect reproductive fitness. We have identified a novel oviposition behavior in *C. elegans* that increases the survival of the young (Lee et al, bioRxv, 2022). In standard laboratory culture, *C. elegans* tend to lay eggs directly on the OP50 with no discernable pattern. However, in a 3D condition, the mothers display a stereotypical behavior, where they temporarily leave far away from the bacterial lawn to lay eggs, resulting in a scattered ring of eggs located outside the bacteria. We showed that this behavior is mediated by low oxygen levels and is regulated by the neuropeptide FLP-17 that is solely expressed in the BAG neuron and its cognate receptor EGL-6 (Lee et al, bioRxv, 2022). Overall, this means an oxygen-sensing circuitry mediated by a neuropeptide and its cognate receptor alters egg-laying behavior to increase survival of the young in toxic environments. Still, the mechanism of how gas sensing regulates 3D oviposition behavior is not known. To better understand this, we used several gas sensing molecule candidates expressed in the BAG neuron such as *gcy-9*, *gcy-33*, *gcy-36*, and *glb-34* to test whether these mutants were important for oxygen-dependent egg-laying behavior. An oxygen-dependent oviposition behavior such as the one described should be adapted to the specific environment and ecology that the nematode inhabits. Along with the laboratory N2 strain, we tested 11 genetically diverse wild strains for oxygen-dependent oviposition behavior and showed variation in oxygen-dependent oviposition behavior. Finally, we show that eggs laid in the *E. coli* lawn in hypoxic conditions were mostly inviable, demonstrating the importance of oviposition behavior in toxic environments on the survival of the young.

1024V **Altered gravity force hinders proper development of multi-dendritic arborization in the PVD neuron in *C. elegans*** Je-Hyun Moon, Jinil LeeDivision of Biological Science and Technology, Yonsei University Mirae Campus

Space flight experiments have shown that altering gravity can affect many biological processes including muscle and bone de-

velopment (Vandenburgh et al,1999). However, gravity's effect on neuronal development is not clear. Previously, we showed that hypergravity affects axonal development of DD/VD motor neurons (Kalichamy et al,2016). Here, we investigate the effects of altering gravity on neuron dendrite development by observing the PVD neuron, a harsh touch sensory neuron, in C. elegans in different gravity conditions. The PVD sensory neuron develops post-embryonically, and by adulthood displays intricately organized and non-overlapping dendrites spanning the entire body length. To investigate whether PVD development is normal in altered gravity, we exposed C. elegans to 100G hypergravity in a centrifuge from egg to young adult. We identified hypergravity-induced abnormal structures in the PVD neuron, particularly 4° branch defects including "L" and "T" shape 4° branch defects. Since basement membrane protein UNC-52/perlecan is known for its fundamental role for patterning PVD 4° branches and increased "L" and "T" shape 4° branch defects (Liang et al , 2015), we exposed *unc-52* mutants to hypergravity. Results show that *unc-52* mutants can suppress hypergravity-induced PVD "L" and "T" shape 4° branch defects. Currently, we are assessing the involvement of SAX-7 in hypergravity-induced dendrite defects. In addition, we exposed *C. elegans* to different gravity forces and gravity time frames and show that PVD 4° branches are affected by 100G hypergravity during 48-72hr time frame which is consistent with the timing of the development of these branches. We also performed *C. elegans* transcriptome analysis and identified putative genes that may be involved in the hypergravity-induced PVD defects. Finally, we are analyzing the development of PVD neurons from *C. elegans* that have been cultured aboard the International Space Station after two spaceflight missions, MME2 project with the European Space Agency in 2021 and Neural Integration System project with the Japan Aerospace Exploration Agency in 2022. This work will offer a fundamental foundation for elucidating how altered gravity affects neuron dendrite development in *C. elegans*.

1025V **RNAi screening of novel dendrite regeneration pathways using an efficient neurite injury method** Pallavi Singh, Kavinila Selvarasu, Mydhily Vasudevan, Anindya Ghosh Roy Cellular and Molecular Neuroscience, National Brain Research Centre

Use of ultrafast laser in the past decades have allowed researchers to perform precise injuries to neurites in various model systems (Yanik et al 2004) for studying the mechanism of neurite regeneration. However, these methods are labor intensive and expensive creating bottlenecks for high throughput assays like RNAi screening for discovering unanticipated pathways. In this study, we have devised an inexpensive and efficient method to injure the PVD dendrites precisely without causing any major health hazards. In this method, a drawn capillary is placed and rolled posterior to the vulva causing a twist and break in the major dendrite of PVD neurons. More than 90% of worms survive this injury and other health parameters like reproductive health and locomotion are unaffected. This protocol allows breaking the primary dendrites of PVD neuron at specific sites with 90% success at the rate of 4-5 worms per minute on the NGM plate itself. This overall speeds up the injury protocol more than ten times in comparison to dendrotomy with lasers. The outcome of twist injury is comparable to laser-induced dendrotomy in terms of length of longest regenerated neurite, branching and self-fusion events. Dendrite regeneration following the twist injury is dependent on pathways such as CED-10 RAC GTPase and AFF-1 as discovered using laser-assisted protocol before (Oren-Suissa et al 2017, Brar et al 2022). This validated our protocol for studying dendrite regeneration using genetic screening. We further discovered that there is a sharp decline in dendrite regrowth potential upon transition from L4 larval stage to day-1 adulthood.

To screen for the genes controlling dendrite regeneration, we developed a RNAi sensitive strain overexpressing *sid-1* in PVD. This injury protocol combined with RNAi allowed screening of 300 genes per month. Using this we have identified dendrite regrowth promoting molecules involving small GTPase RAC-2, integrin subunit PAT-2, and apoptosis inducing factor WAH-1, required for phosphatidylserine externalization in non-apoptotic cells.

This study not only opened up avenue for high throughput neurite injury but also revealed new mechanisms controlling dendrite regeneration.

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1026V **Novel role of *lim-7* on the development of a sleep-controlling neuron in *Caenorhabditis elegans*** Fujia Han, Han Wang Integrative Biology, University of Wisconsin-Madison

Although sleep is a highly conserved behavior throughout phylogeny, the underlying genetic mechanisms of sleep regulation remain poorly understood. In *C. elegans*, behavioral quiescence induced by cellular stress, such as heat shock, is known as Stress-Induced Sleep (SIS). Previous studies of SIS in *C. elegans* have shown that the ALA neuron is the master neuron for this process. However, the molecular and cellular mechanisms underlying the development of this sleep-controlling neuron are still unclear. Our data show that LIM-7, the only *C. elegans* ortholog of human Islet family of LIM-homeodomain transcription factors, plays a key role in ALA cell fate determination and maintenance. Previous literature indicates that loss of *lim-7* is lethal. We discovered a viable allele of *lim-7* that disrupted ALA neuron differentiation and sleep. Transient expression of *lim-7* using a heat shock promoter in either L4 or adult *lim-7* mutants partially rescued the defects in the ALA neuron, suggesting that *lim-7* is essential for ALA neuron fate determination and maintenance. We are testing how *lim-7* works with other transcriptional factors to specify the ALA neuron. We also aim to characterize the transcriptional changes in the *lim-7* mutants. These results can reveal the function of *lim-7* on sleep by controlling the development of the ALA neuron, contributing to a better understanding of sleep.

Keywords: *lim-7*, ALA, Stress-Induced Sleep, transcriptional factor, neural development

1027V **Neural signals that potentiate response to serotonin to modulate neural circuit activity in *C. elegans*** Shavanie Prashad[1], Michael Koelle[21]Yale University, [2]Molecular Biophysics and Biochemistry, Yale University

Serotonin is a neuromodulator that alters the activity and output of neural circuits, but the details of how this occurs are unclear. In the nematode *C. elegans*, serotonin activates the egg-laying circuit by enhancing the response of the vulval type-1 and type-2 muscle cells (vm1s and vm2s) to depolarizing signals so that these muscle cells contract to open the vulva and release eggs. The neurons that release acetylcholine to depolarize the vm2s are known; however, the neurons and signals that depolarize the vm1s remain unidentified. This knowledge gap precludes a detailed understanding of how serotonin potentiates vm1 and vm2 contractions. Using calcium imaging, our laboratory previously showed that the vm1s receive an unknown excitatory signal during each body bend. This signal alone does not evoke coordinated vm1/2 muscle contractions or egg laying unless serotonin is present. We found that the pair of Posterior Ventral Process W (PVW) neurons produce axon branches that terminate in synapse-like varicosities close to the vm1s. These PVW varicosities label with synaptic vesicle and active zone protein markers, suggesting the hypothesis that the PVW neurons release the signal that excites the vm1s. Consistent with the idea that PVW has a role in egg laying, its varicosities exhibit strong calcium transients that begin at each egg release and persist for several seconds. Chemogenetically silencing the PVW neurons did not completely suppress vm1 calcium transients but did block the ability of exogenous serotonin to make animals lay eggs, suggesting that PVW facilitates egg laying in response to serotonin. One idea is that signals from PVW potentiate the response to serotonin to activate the egg-laying circuit. A fascinating aspect of the PVW neurons is that they do not produce any of the seven known small-molecule neurotransmitters in *C. elegans*. Thus, the signal PVW releases to excite its synaptic targets remain a mystery. We hope to identify the unknown signal PVW releases to potentiate serotonin signaling. Our analysis of the cells and signals in the *C. elegans* egg-laying circuit will enable us to understand in unprecedented detail how serotonin modulates neural circuits to drive behavior.

1028V **Hydrogen peroxide positively regulates intestinal neuropeptide secretion during a gut-neural axis mediated oxidative stress response** Qi Jia, Drew Young, Derek Sieburth Zilkha Neurogenetic Institute

Bidirectional signaling between the nervous system and the intestine, the gut-brain axis, is critical for organism-wide homeostasis. We previously found that neurons can activate the antioxidant response in the intestine through the stress-regulated release of the neuropeptide like protein FLP-1 from AIY. Here we report that the release of FLP-1 is controlled in part by signals from the intestine. Acute treatment with the oxidant juglone increases FLP-1 secretion, but juglone-induced FLP-1 secretion is blocked in mutants with impaired intestinal neuropeptide secretion or in the neuropeptide like protein, *flp-2*. Restoring *flp-2* expression selectively in the intestine, but not in the nervous system, rescues the defects in neuronal FLP-1 secretion in *flp-2* mutants. Mutants lacking *flp-2* are hypersensitive to the toxic effects of juglone and have impaired juglone-induced SKN-1 activation in the intestine. The secretion of FLP-2::Venus fusion proteins from the intestine is regulated by hydrogen peroxide (H_2O_2). FLP-2 secretion is increased by acute juglone or H_2O_2 exposure, which elevate levels of H_2O_2 in the matrix of intestinal mitochondria. Impairing the generation of mitochondrial H_2O_2 in the intestine (in mitochondrial *sod-3*/superoxide dismutase mutants) blocks juglone-induced FLP-2 secretion but not H_2O_2-induced FLP-2 secretion. In contrast, elevating endogenous H_2O_2 levels in *prdx-2*/peroxiredoxin mutants increases FLP-2 secretion in the absence of stress. H_2O_2 promotes FLP-2 secretion through *pkc-2*/protein kinase C in the intestine. PKC-2 variants lacking conserved cysteine residues that are oxidized by H_2O_2 fail to restore juglone induced FLP-2 secretion to *pkc-2* mutants. PKC-2 may mediate its effects by phosphorylating the SNARE protein *aex-4*/SNAP25b on T217. In *aex-4* null mutants both baseline and juglone-induced FLP-2 secretion are severely disrupted, whereas *aex-4*(T217A) mutations introduced into the endogenous *aex-4* locus block juglone-induced, but not baseline, FLP-2 secretion. Together our data demonstrate a role for intestinal H_2O_2 in promoting inter-tissue antioxidant signaling through regulated FLP-2 secretion in a gut-neural axis to activate the oxidative stress response.

1029V **Determining the role of the protein kinase C target RIC-4/SNAP25 in ROS-dependent neuropeptide secretion.** Qixin

Zhang, Qi Jia, Derek SieburthZilkha Neurogenetic Institute

Hydrogen peroxide is a signaling molecule generated in mitochondria that can positively regulate neuropeptide secretion from dense core vesicles (DCVs). We previously showed that hydrogen peroxide generated by the mitochondrial superoxide dismutase SOD2 increases secretion of the neuropeptide-like protein FLP-1 from the AIY interneuron, and that the calcium independent protein kinase C family member PKC-1 mediates the effects of hydrogen peroxide on DCV secretion. SNAP-25 is a SNARE protein that mediates the fusion step of exocytosis, and the phosphorylation of SNAP25 on a conserved serine residue by PKC has been implicated in positively regulating exocytosis in a number of systems. Here we test whether the worm SNAP25 ortholog, RIC-4 is a target of PKC-1 phosphorylation in promoting peroxide-induced FLP-1 secretion. Acute treatment with the mitochondrial toxin juglone leads to increased FLP-1 secretion from AIY, as measured by increased coelomocyte fluorescence of FLP-1::Venus-expressing animals. We found that a reduction-of-function *ric-4* mutation decreases baseline FLP-1 secretion and eliminates juglone-induced FLP-1 secretion. Expression of wild type *ric-4* cDNA fully restores normal baseline and juglone-induced FLP-1 secretion to *ric-4* mutants. Expression of a *ric-4* variant in which the putative phosphorylated serine has been mutated to alanine (ric-4(S189A)) restores baseline FLP-1 secretion but fails to restore juglone-induced FLP-1 secretion to *ric-4* mutants. Similarly, introducing the S189A substitution into the endogenous *ric-4* locus using CRISPR/Cas9 results in no change in secretion of FLP-1 under non-stressed conditions, but significantly impairs juglone-induced FLP-1 secretion. Thus, the phosphorylation of RIC-4 on Ser189 may be important for FLP-1 exocytosis specifically during oxidative stress. Because the ric-4(S189A) substitution does not eliminate juglone-induced FLP-1 secretion, we propose that PKC-1 may phosphorylate RIC-4 as well as additional as-yet unidentified targets to control stress-induced DCV exocytosis.

1030V **A candidate of GPCR-type thermoreceptor involved in heat tolerance of *C. elegans*** Chinatsu Morimoto[1], Chie Miyazaki[1], Kohei Ohnishi[1], Tohru Miura[1], Akane Ohta[1], Astushi Kuhara[21]Graduate school of Natural Science, Inst. for Integrative Neurobio., Konan univ., Japan, [2]Graduate school of Natural Science, Inst. for Integrative Neurobio., Konan univ., Japan, PRIME, AMED

Temperature is essential for animals to survive. To identify novel mechanisms of animal thermosensation, we are analyzing temperature acclimatization and tolerance of nematode *C. elegans*, as a simple model. In cold tolerance, wild-type animals which were cultivated at 15°C can survive at 2°C, while 25°C-cultivated wild-type can not survive at 2°C. Also, we are analyzing heat tolerance with temperature acclimatization; 25°C-cultivated wild- type can survive at 31°C, whereas 15°C-cultivated animals can not survive at 31°C. Temperature signaling for cold tolerance are transducted via trimeric G protein in thermosensory neuron (Ohta et al., *Nature commun*, 2014; Kuhara et al., *Science*, 2008), however a GPCR-type thermoreceptors that could function upstream of the G proteins have not been found. We performed exhaustive RNAi screening for about 1000 GPCR genes, and found that 86 GPCRs were involved in cold tolerance. Among them, we are analyzing about *srx* gene because *srx* knock-out mutant showed significantly increased-cold tolerance, in this study. However, we found that abnormal cold tolerance of *srx* mutant was caused by second mutation in mutant genome, and found that its responsive gene was a *snt-2* gene encoding synaptotagmin. Therefore, we constructed additional knockout mutants of *srx* gene by using CRISPR-Cas9, and isolated knockout alleles, but all of them were not abnormal in cold tolerance. We then tested various temperature-related assay, we found that *srx* mutant showed abnormally increased-heat tolerance. To determine the expression pattern of SRX, we used a whole neuron multicolor map strain called NeuroPAL, and SRX was expressed in an chemosensory neuron. SRX was localized at the sensory cilium and dotted at axon and cellbody of the neuron at a head of wild-type. By contrast, SRX::GFP was not localized at sensory cilium and was accumulated at the cell body in *unc-101* mutant impairing clathrin adaptor that transports proteins to dendrite specifically. To determine whether the chemosensory neuron is responsive to temperature stimuli, we introduced calcium imaging. We found that Ca2+ concentration in the neuron of wild-type was increased upon warming. To investigate whether SRX is responsive to temperature, we are attempting to ectopically expressing GPCR SRX in non-warm sensitive gustatory neuron ASER and culture cell.

1031V **Geraniol protects against oxidative stress and proteotoxicity in *Caenorhabditis elegans* Parkinson›s disease models** Stéfano Romussi[1], Natalia Andersen[1], Sofía Ibarguren[2], Diego Rayes[1], María José De Rosa[11]Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET). Departamento de Biología, Bioquímica y Farmacia (UNS), [2]Departamento de Biología, Bioquímica y Farmacia (UNS)

Due to the increase in life expectancy worldwide, age-related disorders such as neurodegenerative diseases have become more prevalent. Elevated levels of oxidative stress could modulate the progression of neurodegenerative diseases. For example, in Parkinson's disease it has been shown that compromising the capacity to scavenge free radicals can exacerbate α-synuclein (α-syn) aggregation and proteotoxic damage.

Geraniol, a plant-derived essential oil, has recognized antioxidant properties. Considering that oxidative stress contributes to proteotoxic disease progression, compounds with antioxidant activity have been postulated as potential therapeutic agents. *C.*

elegans is widely used in biomedical research. There is a high level of homology between *C. elegans* and mammalian genes (including proteins involved in cytoprotective mechanisms). In fact, several neurodegenerative diseases can be recapitulated in this animal.

In this work, we use *C. elegans* Parkinson's disease models to evaluate the *in vivo* effect of geraniol. We found that geraniol improves impaired locomotion in these animal models. Interestingly, geraniol also decreases α-syn aggregation. In addition, our last preliminary results suggest that DAF-16 is not involved in geraniol activity. So far, these results indicate a potential antiproteotoxic effect of this drug in *C. elegans* Parkinson's disease models. Therefore, we propose to combine genetic, microscopy and behavioral techniques to unravel geraniol effect in *C. elegans* neurodegenerative diseases models.

These studies could provide a proof of concept of the potential of geraniol as a promising compound to retard proteotoxic diseases.

1032V **Propionate Regulates the Sexual Motivation through Gut-brain Axis in *C. elegans* Males** Yi Sin Wang, Chun Hao Chen
Institute of Molecular and Cellular Biology, College of Life Science, National Taiwan University

Microbiomes have been shown to modulate the host's motivations through gut-brain interactions, whereas underlying neural mechanisms are poorly understood. Here, we investigate the effect of bacterial diets on the sex drive of *C. elegans* males. Males reared on *Escherichia coli* OP50, the standard diet in the laboratory, display mate-searching behavior by leaving food sources. By contrast, males reared on a bacterial diet *Comamonas sp.* DA1877 significantly reduce the leaving tendency, while the locomotor activity and the food preference are preserved. These observations suggest that bacterial diets affect the sex drive in males. Interestingly, odors, water-soluble molecules, and vitamin B12 from DA1877 are dispensable. In addition, reducing sex drive requires exposure to limited live DA1877 mixed with OP50 at the adult stage, demonstrating a dominant effect on motivational changes. We find that the motivational changes by DA1877 depend on the presence of a short-chain fatty acid propionate in the host, as the accumulation of propionate in the *pcca-1* and *acdh-1* mutants that disrupt the propionate breakdown pathway and exogenous supply of propionate on the DA1877 diet restore the sex drive in males. Through a screen of neurotransmitter mutants, we identify that octopamine is required and sufficient to modulate the mate-searching behavior, providing a neural pathway that modulates the motivation in males. Lastly, the effect of DA1877 on motivational changes is evolutionarily conserved in androgynous *C. remanei*. Our study thus illustrates how the microbiome regulates the sexual motivation in *C. elegans* males by modulating host metabolism and octopaminergic pathways, which endows a paradigm to gain mechanistic insights into intricate gut-brain interactions for motivational controls.

1033V **The metabotropic glutamate receptor homologs MGL-1 and MGL-2 are key for sensing nutritional status in *C. elegans*** Ailin Lacour[1], Maria Gabriela Blanco[2], Agustina Zabala[1], María José De Rosa[1], diego hernan rayes[11]Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia (CONICET-UNS), [2]Instituto de Investigaciones Bioquímicas, Departamento de Biología, Bioquímica y Farmacia de Bahía Blanca (CONICET-UNS)

The mechanisms that allow the nervous system (NS) to sense nutritional state and adapt animal behavior are poorly understood in most species. The simplicity of its NS and its known connectome make C. elegans a useful system to study these mechanisms. Results from our laboratory showed that inhibition of the tyraminergic neuron RIM during fasting, enhances serotonin release from other neurons when the animal reencounters food, allowing it to slow down locomotion and start feeding. Mutations in the GPCRs, MGL-1 and MGL-2, located in two presynaptic interneurons to RIM have been reported to induce autophagy even in well-fed animals. Here, we performed behavioral assays on mgl-1; mgl-2 double mutants. We found that these animals, even when well fed, show a significant decrease in locomotion when they find food, similar to fasted wild-type animals. Moreover, when we exposed these mutants to GFP-expressing bacteria, the fluorescence in the intestine is higher than that of wild-type animals, suggesting a higher feeding rate. These initial results suggest that the metabotropic receptors MGL-1 and MGL-2 are key for satiation sensing. We propose, therefore, to determine what these satiety signals are and the neuronal circuits involved. Given that this behavioral plasticity modulated by the nutritional state is observed throughout the animal kingdom, and that several fundamental processes are highly conserved, these results may provide universally relevant information.

1034V **Effects of temperature on mechanosensation and neurodegeneration** victoria c collio[1], Juan pablo Castillo[2], Andrea Calixto[21]science faculty, Universidad de Valparaíso, [2]Universidad de Valparaíso

Mechanotransduction is a fundamental process underlying the senses of touch, balance, proprioception and hearing. Mechanosensory channels have been identified in bacteria, yeast, insects and vertebrates, belonging to various superfamilies. In *C. elegans*, MEC-4, a DEG/ENaC family protein is the pore forming unit of the mechanosensory channel, expressed in the Touch Receptor Neurons (TRNs). Accessory proteins to the channel are MEC-2, associated with the inner leaflet of the plasma membrane and MEC-6, located in the extracellular domain. A gain of function mutation (A713V) in MEC-4 located in residues near trans-

membrane segment 2 (MEC-4d), causes the unregulated entry of Na^+ and Ca^{2+} and the concomitant degeneration of the TRNs. Previous unpublished observations from our group revealed that gentle touch response and TRN degeneration are affected by temperature changes in the range of 15 to 25°C, such that warmer temperatures both decreases responsiveness of the nematode and that TRNs bearing the MEC-4d channel are protected from premature degeneration. Our results show that the response to gentle touch in wild type nematodes maintained at 25°C is diminished (5/10) compared to 20°C (9/10) or 15°C (10/10). At the same time the neurodegeneration rate is lower at 25°C with functional axons reaching 94% after 96 hours, compared to 15°C where animals have fully degenerated axons, consistent with the constitutively open MEC-4d channel. These results suggest that the complex channel is sensitive to environmental temperature. Effects of temperature on DegENaC channels have not been reported and we do not know where does the temperature sensitivity comes from. In the future we expect to test the different subunits dependence on temperature by heterologous expression of the complex in Oocyte eggs and by direct patch clamp on touch cells in culture.

1035V **The role of gap junction molecule inx-19 in post-embryonic post-mitotic neuronal maturation** Molly Reynolds[1], HaoSheng Sun[21]Cell, Developmental Integrative Biology, University of Alabama at Birmingham, [2]Cell, Developmental, Integrative Biology, University of Alabama at Birmingham

While the molecular mechanisms controlling early neurodevelopmental events like neuronal specification and migration have been the focus of much research, the molecular mechanisms controlling the maturation of the post-mitotic nervous system from birth to adulthood are not well understood. Electrical synapses are composed of gap junctions, created from connexins in chordate and innexins in non-chordate animals. Although the dynamic developmental expression of connexins/innexins have been suggested to play important roles in post-mitotic brain maturation, the mechanisms by which they control neuronal maturation and by which their dynamic developmental expression is regulated are largely uncharacterized. We used *Caenorhabditis elegans* (*C. elegans*) as our experimental model due to their stereotyped and fast developmental cycle, invariant cell lineage, and the ability to identify the expression pattern of any molecule in the entire nervous system to single neuron resolution. We approach these questions by first identifying the dynamic expression of the gap junction molecule, *inx-19*, nervous-system wide in single neuron resolution across post-embryonic development nervous-system-wide. Next, we characterized the role of *inx-19* in locomotor behavior across development. To explore the mechanisms controlling the dynamic *inx-19* expression, we are investigating the roles of both genetic timer mechanisms and neuronal activity. To study genetic timer mechanisms, we are first using a candidate approach to examine members of the heterochronic pathway. To examine the role of neuronal activity, we are employing histamine chloride (HisCl) tool to examine whether inhibition of neuronal activity at specific developmental time periods affect dynamic *inx-19* expression across development. Identifying the regulation and role of *inx-19* will allow us to better understand how dynamic gap junction expression across development regulates neuronal maturation, as well as uncover how dysregulation of these mechanisms may govern neurodevelopmental diseases.

1036V **Inflammation's Affect on Regulation of ADM-4 and Downstream Targets of Notch Signaling** Julia Zickus Biology, Elmhurst University

Neurodegenerative diseases share the common feature of slow, progressive destruction to the central nervous system (CNS), however, the mechanistic onset of these diseases remains unknown. Recently, inflammation of the brain tissue due to cytokine proteins has been associated with neurodegeneration. Exposure to pro-inflammatory cytokine Interleukin-6 showed a significant decrease in learning in *Caenorhabditis elegans.* C. elegans have behavioral plasticity and a learning index has been established, allowing these to be used as a model organism of study for neurodegeneration. To further understand the mechanism underlying this decreased learning, the Notch1 signaling pathway was chosen to be further analyzed. This pathway is conserved in *C. elegans,* and responsible for neurogenesis, something no longer observed in neurodegenerative diseases. Also, mutations within the Notch1 signaling pathway were found to accumulate plaques seen in the brain of Alzheimer's patients. Notch1 regulatory gene Adm-4 was quantified using fluorescence and seen to have significant decreased expression in the cytokine-exposed *C. elegans.* Given a decreased expression of the Adm-4 regulatory gene after cytokine exposure, there is likely misregulation of Notch1. Downstream targets of Notch1 signaling were quantified. A further understanding of the mechanism underlying neurodegeneration can open more opportunities to treat these irreversible neurocognitive diseases.

1037V **Vanilloids impedes the nocifensive response of *Caenorhabditis elegans* to noxious heat, and proteomics revealed specific signaling and metabolic pathway are involved** Nkambeu Bruno[1], Nkambeu Bruno[2,3], Ben Salem Jennifer[2], Beaudry Francis[21]Veterinary biomedicine, University of Montreal - Faculty of veterinary science, [2]University of Montreal - Canada, [3]Biomedecine, University of Montreal

Introduction

Vanilloids including capsaicin and eugenol are ligands of the transient receptor potential channel vanilloid subfamily member 1

(TRPV1). Prolonged treatment with vanilloids triggered the desensitization of the TRPV1 leading to analgesic or antinociceptive effects. Following *C. elegans* genome sequencing, several genes encoding TRP ion channels, including TRPVs, were identified. Furthermore, several studies have shown that *C. elegans* TRPV orthologs (OSM-9 and OCR-2) are associated with behavioral and physiological processes, including sensory transduction. We have already shown capsaicin and eugenol targets *C. elegans* TRPV orthologs. The objective of this study was to perform proteomics to identify the proteins and pathways responsible for the induced phenotype.

Methods

N2 (Bristol) and other strains were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota (Minneapolis, MN, USA). Strains were maintained and manipulated under standard conditions. Capsaicin or eugenol was dissolved in Type 1 Ultrapure Water at a concentration of 25 µM. *C. elegans* was isolated and washed and then expose to capsaicin or eugenol for 60 min. Then after, the nematodes were isolated, carefully washed, homogenized and protein were extracted, normalized, denature, reduced, alkylated, and digested with trypsin. Ultra-high-performance liquid chromatography/Quadrupole-Orbitrap mass spectrometry analysis operating in Data-Dependent Acquisition (DDA) mode was performed to identify and quantify proteins. Pathway analyses were performed using Metascape and the Reactome database.

Preliminary data

Capsaicin and eugenol can impede nocifensive response of *C. elegans* to noxious heat (32°C – 35°C) and the effect was reversed 6h post exposition. Additionally, we have identified the capsaicin target, OCR-2 and eugenol act redundantly with both OSM-9 and OCR-2. After we use proteomic investigations to performed *C. elegans* exposed to vanilloids. Preliminary results demonstrate that several specific processes were modulated following the pharmacological manipulation of *C. elegans* with capsaicin and eugenol. The inflammatory signaling pathways and the regulation of translation stand out from our bioinformatics analyses. These two processes are intimately linked to cell protection and survival mechanisms.

Novel aspect

Proteomics reveals inflammatory signaling pathways are triggered by the agonistic effects of capsaicin and eugenol on *C. elegans* vanilloid receptors.

1038V **The Ketone Body β-hydroxybutyrate ameliorates neurodevelopmental deficits in the GABAergic system of *daf-18/PTEN Caenorhabditis elegans* mutants.** Sebastián Giunti[1,2], María Gabriela Blanco[1,2], María José De Rosa[1,2], Diego Hernán Rayes[1,21]Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia (CONICET - UNS), [2]Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur

An increased Excitation/Inhibition (E/I) ratio in the brain is a hallmark of neurological disorders such as Autism Spectrum Disorder (ASD). Mutations in *PTEN,* a gene that encodes for the main negative regulator of the conserved PI3K/AKT pathway, are strongly associated with ASD. However, it is unclear how *PTEN* deficiencies can lead to E/I disequilibrium. The *C. elegans* neuromuscular system, where both excitatory (Cholinergic) and inhibitory (GABAergic) motor neurons regulate muscle activity, provides a proven simple model for studying E/I balance. We found that mutants in *daf-18* (ortholog for *PTEN)* exhibit phenotypes typical of animals with deficient GABAergic signaling. While cholinergic neuron morphology is normal, we observed defects that occur specifically in GABAergic neurites. This selective impairment accounts for the disruption of the E/I balance in *daf-18* mutants. In addition, we showed that the low activity of the transcription factor DAF-16 (ortholog for FOXO3A) during GABAergic neurodevelopment arises for the behavioral defects in *daf-18* mutants. Ketogenic Diets (KGDs), in which the production of ketone bodies (KBs) is forced, have been established as an effective treatment for disorders associated with E/I imbalances. The mechanisms underlying its effect are not understood. We found that exposure to the KB hydroxybutyrate (βHB) during early development improves GABAergic neurodevelopment in *daf-18* mutants. This effect depends on DAF-16/FOXO. Since the PI3K/AKT pathway is highly conserved, this study may provide universal information on the proven link between PTEN mutations and neurodevelopmental defects and, equally important, the mechanisms underlying KGDs positive effects on neuronal disorders characterized by E/I imbalance.

1039V **Reconstruction of *C. elegans* locomotion by optimal fluid control** Yongxing Wang, Thomas Ranner, Netta CohenUniversity of Leeds

C. elegans lives in a 3D environment whose locomotion however has been studied primarily through a microscope on a flat dish. There has been progress in developing a full 3D model of the worm, such as OpenWorm [1] or body midlines modelled as 3D curves [2,3]. Recent advances in imaging and midline reconstructions of worm's locomotion in 3D have opened up questions about the underpinning mechanics and neuromuscular control in 3D [4]. This dataset provides accurate body-midlines but

dorsal-ventral orientations can not be recovered. We use a modelling approach to infer beyond what can be directly observed through the data.

The aforementioned problem may be formulated as an optimal control problem for the evolution of a mechanical rod with the control force and torque being interpreted as the reaction force, activated by the worm muscles, from the surrounding fluids [5]. Additionally, we present a new embodied 3D model combining internal body mechanics, muscles and external fluid flow. The model minimises the viscous dissipation of the flow by controlling muscles on the worm's body surface but constrained to match data for the midline velocity. We show the advantages of this model by comparing with our previous rod model as well as laboratory video footage.

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1040V **Neural modulation of systemic stress response requires the insulin like-peptide INS-3** Tania Veuthey[1], Sebastian Giunti[2], Maria Jose De Rosa[1], Mark Alkema[3], Diego Rayes[11]Invertebrate Neurobiology Laboratory, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), [2]invertebrate neurobiology laboratory, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), [3]Department of neurobiology, UMass Chan Medical School

Throughout the animal kingdom, the perpetuation of the flight response leads to reduced ability to cope with environmental challenges, a drastic lifespan reduction, and an increase in disease susceptibility. We showed that, in *C. elegans*, the tyraminergic neuron RIM supplies a state-dependent neural switch between acute flight and long-term environmental stress responses. During the flight-stress response RIM neurons release TA, which stimulates the intestinal adrenergic-like receptor TYRA-3. This leads to DAF-2/Insulin/IGF-1 pathway activation and inhibition of cytoprotective mechanisms in the intestine and other tissues. We hypothesized that TYRA-3 stimulates the release of Insulin-Like Peptides (ILPs) from the intestine that can systemically activate the DAF-2 insulin/IGF1 receptors. We focused on strong agonists ILPs that are expressed in the intestine (INS-3, -4, -6, -32, and DAF-28). We found that *ins-3* mutants are resistant to both heat and oxidative stress, much like *tyra-3* mutants. Moreover, *ins-3* mutants are resistant to the impairment of stress resistance upon exposure to exogenous tyramine. In addition, *ins-3;tyra-3* double mutants are as resistant to environmental stress as single mutants, suggesting that both genes act in the same pathway. Since *ins-3* is expressed in neurons and the intestine, we performed tissue-specific rescue experiments. We found that expression of *ins-3* in the intestine restores stress resistance to wild-type levels. Taken together, our results suggest that intestinal activation of TYRA-3 by the escape neurohormone TA leads to INS-3 release which acts as an endocrine, autocrine, and/or paracrine signal to activate DAF-2 in different tissues.

1041V **Bacterial diets are able to modulate life-history treats in *C. elegans* models of neurodegenerative diseases** Tania Veuthey[1], Andreas Burkovski[21]Invertebrate Neurobiology Laboratory, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), [2]Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg

As life expectancy increase, age-related disorders, such us neurodegenerative diseases (ND), have become more prevalent. Moreover, treatments only attenuate some symptoms, but fail to arrest characteristic neuronal proteotoxicity. Thus, new challenges emerge to science in order to understand molecular basis of these disorders. Lately, the gut-brain axis has gain attention and a close relation between gut microorganism and ND has been proposed. The aim of our work was to evaluate the relevance of the microbiota in the progression of proteotoxic-based disorders, assessing the impact of six non-pathogenic bacterial diets on life-history traits in *C. elegans* models of ND (vs standard OP50). In a first approach, we found 2 bacteria, *Escherichia coli* K12 and *E. coli* HB101, able to improve locomotion in liquid media, in worm's model of Parkinson disease (PD) at adult day 4, versus *E. coli* OP50. Moreover, an age-dependent locomotion improvement, between larva-L4 and adult day 4, was observed in solid media after feeding PD model´s worms with 4 different bacteria versus *E. coli* OP50. We also observed an increase in

the developmental timing of wild-type worms grown in 4 bacteria versus *E. coli* OP50, but more interesting was the accelerated developmental rate selectively found in models of PD and Huntington disease feed with *E. coli* BL21 (DE3). We are currently evaluating aggregate numbers, lifespan and mitochondrial morphology among others. Our results allowed us to identify bacteria with the ability to drive physiological outcomes and improve health status of *C. elegans* models of neurodegenerative diseases.

1042V **Low abundance of propionate promotes α-synuclein-induced neurodegeneration in *C. elegans* through intestine-neuron signaling** chenyin wang[1], Chaogu Zheng[21]School of Biological Sciences, The University of Hong Kong, [2]The University of Hong Kong

Our previous work identified pro-neurodegenerative bacterial genes using the *Caenorhabditis elegans* PD models with neuronal expression of human a-synuclein. Several of those pro-neurodegenerative genes code for proteins involved in the synthesis of vitamin B12 in *E. coli*. We found that depletion of B12 from the *E. coli* diet ameliorated the PD symptoms in *C. elegans,* suggesting that B12 may exacerbate neurodegeneration. Since that dietary B12 is known to breakdown propionate (a short-chain fatty acid) in *C. elegans*, we hypothesize that B12 may promote neurodegeneration by reducing the level of propionate, which has been shown to be neuroprotective in several PD models. We found that propionate supplementation could indeed protect neurons from a-synuclein proteotoxicity. Moreover, transcriptomic analysis found that B12-downregulated genes are also downregulated in PD animals compared to wild-type animals, indicating that dietary B12 and neuronal a-synuclein aggregation target the same genes, both likely through the downregulation of propionate. In fact, our GC-MS analysis found that PD animals indeed have a lower level of propionate than wild-type animals. Propionate serves as a signaling molecule that controls a range of downstream genes, including the transcription factor *nhr-68*, which is significantly downregulated in PD animals compared to the wild type. Interestingly, rescuing *nhr-68* expression in the intestine but not in neurons could rescue neurons from degeneration, suggesting inter-organ signaling between neurons and the intestine. Furthermore, neuronal a-synuclein overexpression induces the activation of mitoUPR markers in the intestine, which contributes to the reprogramming of metabolic genes in the intestine. Overall, our studies suggest that PD animals experience a vitamin B12-like reprogramming of intestinal metabolism due to the low abundance of propionate; this reprogramming leads to reduced energy production in the intestine, which promotes neurodegeneration.

1043V **Turning related neurons RIV, SMB, and SAA gates behavior context dependent processing of mechanosensory stimuli in *C. elegans*** Sandeep Kumar[1], Anuj K. Sharma[2], Andrew M. Leifer[1,21]Princeton Neuroscience Institute, Princeton University, [2]Physics, Princeton University

How does the nervous system integrate external sensory stimuli and the animal's current behavior state to give rise to an appropriate motor response? To answer this question, we investigate the *C. elegans* response to touch because our previous results have shown that worms are less likely to reverse when a gentle touch stimulus is delivered during turns than during forward movement [1].

To probe where in the network behavior context arises, we probed interneurons AIZ, AIB, RIM, AVE, and AVA that are downstream of mechanosensory neurons. We expressed excitatory opsins in these neurons and used a high throughput method [2] to activate each neuron as the animal moved forward or turned and measured the evoked behavioral response. We found that activating neurons AIZ, AIB, RIM, and AVE evoked reversals with a lower probability during turns compared to activation occurring when the animal moved forward. In contrast, activating neuron AVA evoked reversals with similar probability regardless of whether activation occurred when the animal was moving forward or turning. This led us to hypothesize that inhibitory signals during turns are integrated somewhere upstream of neuron AVA.

We next sought to investigate the potential source of this hypothesized inhibition. Wang et al., [3] previously showed and we independently confirmed that inhibition of turning associated neurons RIV, SMB, and SAA increases reversal duration. This led us to hypothesize that these neurons could be the potential source of gating during turns. To test this hypothesis, we simultaneously activated touch neurons and inhibited neurons RIV, SMB, and SAA during turns and found that the probability of evoked reversal is similar to that evoked when the animal moves forward. These findings are consistent with our hypothesis that neurons RIV, SMB, and SAA act as a gate by potentially sending inhibition to the network somewhere upstream of neuron AVA, thus preventing mechanosensory information from traveling downstream in the network.

References:

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2. Liu et al., 2022, PLoS Biology.
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1044V **The synergistic role of tau and α-synuclein in neurodegeneration and cognitive decline in both Alzheimer's and Parkinson's diseases** Julie E Vincent[1], Danielle E Mor[21]Medical College of Georgia at Augusta University, [2]Neuroscience and Regenerative Medicine, Medical College of Georgia at Augusta University

Alzheimer's disease (AD), followed by Parkinson's disease (PD), are the most common age associated neurodegenerative disorders, both with no cure or disease-modifying treatments. AD is primarily defined by pathologies of amyloid-b and tau proteins, while PD is defined by α-synuclein (α-syn) protein deposits. However, these pathologies often coexist in AD and PD and may potentially act synergistically to promote neuron degeneration. Cholinergic neurons in the basal forebrain degenerate in both AD and PD, and the loss of these cells is thought to contribute to cognitive decline. Yet, it remains unknown what causes protein aggregation and how this leads to neuronal death and cognitive dysfunction. Mitochondrial dysfunction is likely a key player in AD and PD pathogenesis via intracellular transport defects specifically linked to α-syn and tau accumulations. I propose to test the hypothesis that tau and α-syn synergistically cause severe mitochondrial dysfunction leading to neurodegeneration and cognitive decline in both AD and PD contexts using the rapidly aging and highly manipulable *C. elegans* model system. Transgenic strains expressing pan-neuronal human α-syn, tau, or a α-syn;tau double transgenics are being tested in learning and memory assays. Compared to non-transgenic controls, a-syn alone only showed mild learning and memory deficits. Similarly, tau alone caused mild or no effects on learning and memory. However, when combined, α-syn and tau eliminate learning and memory function, consistent with synergistic toxicity leading to cognitive deficits. To determine the mechanisms by which a-syn and tau induce cognitive decline, we are isolating cholinergic neurons from the disease strains and will perform RNA-sequencing, identifying mitochondrial genes and their expression changes. Cholinergic health and mitochondrial function are also being evaluated using imaging, behavioral experiments, and Seahorse respirometry assays. The results of this work will shed light on the mechanisms of cholinergic neurodegeneration and identify potential therapeutic targets to treat AD and PD cognitive decline.

1045V **Glutamate signaling mediates *C. elegans* behavioral plasticity to pathogens** Chun-Ying Yu[1], Howard Chang[21]National Chung Cheng University, [2]Rowan University SOM

In *Caenorhabditis elegans*, sensory neurons mediate behavioral response to pathogens. However, how *C. elegans* intergrades these sensory signals via downstream neuronal and molecular networks remains largely unknown. Here, we report that glutamate transmission mediates behavioral plasticity to *Pseudomonas aeruginosa*. Deletion in VGLUT/*eat-4* renders the mutant animals unable to elicit either an attractive or an aversive preference to a lawn of *P. aeruginosa*. AMPA-type glutamate receptor GLR-1 promotes the avoidance response to *P. aeruginosa*. SOD-1 acts downstream of GLR-1 in the cholinergic motor neurons. SOD-1 forms a punctate structure and is localized next to GLR-1 at the ventral nerve cord. Finally, single-copy ALS-causative *sod-1* point mutation acts as a loss-of-function allele in both pathogen avoidance and *glr-1* dependent phenotypes. Our data showed a link between glutamate signaling and redox homeostasis in *C. elegans* pathogen response and may provide potential insights into the pathology triggered by oxidative stress in the nervous system.

1046V **Indole glucosides act as a gut-to-brain signal to drive microbially-influenced locomotory escape responses** Julia Balch[1], Chester Wrobel[2], Madhumanti Dasgupta[1], Jingfang Yu[2], Frank Schroeder[2], Michael O'Donnell[11]Department of Molecular, Cellular, and Developmental Biology, Yale University, [2]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University

Recent advances in the study of animal-microbiome interactions demonstrate that inter-kingdom signaling occurs between bacteria and host nervous systems. In C. elegans, well-developed genetic and behavioral techniques as well as gnotobiotic culture allows for the mechanistic understanding of microbe-nervous system signaling. Previous work has identified indole, a common bacterial tryptophan metabolite, as a neuroactive compound potentially involved in microbe-host nervous system signaling in diverse animal systems. However, it is unclear how gut microbial indole metabolites are trafficked to and sensed by the nervous system. In C. elegans, bacterially produced indole is incorporated into an array of carboxylesterase-diversified modular glucosides (MOGLs) produced in the lysosome-related organelles (LRO) of the intestine. We find that disruption of indole MOGL (iglu) biosynthesis via elimination of bacterial indole production or prevention of LRO formation results in behavioral defects in the locomotory escape response. Additionally, expression of the carboxylesterase CEST-1.2, which is responsible for the 2' acylation of iglus, in both the gut and olfactory neurons is necessary for producing sustained reversals in response to anterior touch. We propose a model by which the assembly of iglus from bacterial metabolites in the intestine are trafficked to the nervous system and hydrolyzed to release free indole. The neuronally expressed transient receptor potential ankyrin 1 (TRPA-1) channel is a putative target for neuronally released indole to act on locomotory behavior circuits. This system suggests that glycosylation of bacterial metabolites may represent a general mechanism by which microbiota regulate the nervous systems of their animal hosts.

1047V **Cool worms delay forgetting – Bi-Polar Switch For Turning Forgetting ON and OFF In C. elegans Nematodes** Dana Landschaft Berliner[1], Guy Teichman[2], Sarit Anava[2], Hila Gingold[2], Vladyslava Pechuk[3], Yehuda Salzberg[3], Meital Oren-Suissa[3], Oded Rechavi[11]Neurobiology, Tel Aviv University, [2]Tel Aviv University, [3]Weizmann Institute of Science

Despite being a crucial element of brain function, the processes that regulate the formation and retention of memories are not yet fully comprehended. Similarly, the phenomenon of forgetting, which allows living beings to adapt to changing circumstances, remains elusive. In the early 20th century, considerable attention was devoted to the question of whether forgetting is an active or passive process. The active interference theory, which proposes that forgetting is an active phenomenon, has gained widespread acceptance as a general framework of memory loss. However, the underlying mechanisms that govern the kinetics of forgetting are still not well understood.

The Caenorhabditis elegans nematode has a very simple nervous system composed of only 302 neurons. Yet, it can form associative memories. These memories, however, tend to fade quickly - at normal cultivation temperatures, worms typically forget associations between attractive odors and starvation after 2-3 hours.

In this work, we discovered that worms are able to delay forgetting for as long as they are cooled down on ice and once the temperatures return to normal, the worms start forgetting again. We show that environmental induction of cold tolerance led the worms to lose their ability to delay forgetting when cooled. Therefore, we hypothesize that this phenomenon is governed by biological regulation rather than a general decrease in enzymatic activity. By combining whole-worm RNA sequencing and behavioral assessment, we identified multiple genes in the diacyl glycerol (DAG) pathway which are required for delayed forgetting on ice. Through exploring the relationship to the cold tolerance pathways, we found that treating worms with lithium, a drug used for decades to treat bipolar disorder, delays forgetting at regular temperatures and only in cold-sensitive worms. To summarize, we examined various components of the memory system that can be manipulated to postpone the process of forgetting.

1048V **Suppression of Parkinson's disease in *C. elegans* by the probiotics isolated from fermented foods through the gut-nerve ring connection** Seunghun Kang[1], Seungeun Lim[1], Jiaoyang Li[2], Shin Sik Choi[11]food and nutrituon, Myongji University, [2]Energy Science and Technology, Myongji University

Parkinson's disease (PD) is a neurodegenerative disease caused by the gradual destruction of dopamine nerves in the part of the brain called the substantia nigra. Many researches have recently shown that gut microbiome regulates PD through the gut-brain axis and short-chain fatty acids (SCFAs). In this study, the neuroprotective effect of specfic lactic acid bacteria (LAB) strains from fermented food was investigated using a soil nematode *Caenorhabditis* elegans model, regarding the connection of intestine-nerve ring system. We found that the selected LAB strains producing higher concentration of SCFAs were colonized in the intestines of *C. elegans*. Dietary LAB strains efficiently prevented neurodegenerative locomotive behaviors and altered the expression level of several genes related with dopamine neurons in *C. elegans*. These results demonstrate an anti-PD effect of probiotic bacterial strains through the SCFAs-involved gut-brain connected mechanism.

1049V **Electronic stimulation as a treatment for Parkinson's disease using *C. elegans* model system** SEUNGEUN LIM[1], Jiaoyang Li[2], Hai Anh Thi Le[3], Yong Tae Park[3], Shin Sik Choi[41]Food and Nutrition, Myongji University, [2]Energy Science and Technology, Myongji University, [3]Mechanical Engineering, Myongji University, [4]Food and Nutrition, Energy Science and Technology, Myongji University

Parkinson's disease (PD) is a neurodegenerative disease led by the demolition of dopamine neurons in the brain substantia nigra. Although PD treatments including dopamine agonists, exercise, diet, and deep brain stimulation have been developed, those showed low efficiency or efficacy along with invasiveness and expensiveness. In this study, we investigated an application of electronic stimulation (ES) device which was based on the action of triboelectric nanogenerator for the PD model using *Caenorhabditis elegans*. According to the results from lifespan, body size and reproduction ratio, the ES did not show any biological toxicity in the wild-type N2 strains. The mutant worms with less biosynthesis of dopamine efficiently improved the dopaminergic behavior of basal slowing response by ES treatment. In addition, ES device treatment significantly recovered the defective dopamine neurons in the PD-induced muatnt worms, and significantly diminished the aggregation of human alpha-synuclein, known to be a biochemical hallmark of PD, in the *C. elegans* model. These results demonstrate that ES device has a potential of the therapeutic application for PD.

1050V **Molecular analysis of synapse elimination in GABAergic DD motor neurons** Samuel Liu, Kellianne Alexander, Julia Russell, Michael M FrancisNeurobiology, University of Massachusetts Chan Medical School

A critical step in brain development is the rewiring of synaptic connections toward the formation of mature neural circuits. While some connections are formed or stabilized during nervous system maturation, others are eliminated. Though disruptions in the developmental remodeling of human neural circuits are linked with neurological disorders such as schizophrenia and autism spectrum disorders, we have an incomplete understanding of the molecular mechanisms directing this process. My work investigates molecular mechanisms of synaptic remodeling using the nematode *Caenorhabditis elegans* as a model. The compact and invariant organization of the *C. elegans* nervous system in combination with the genetic tools available make it a powerful system for pursuing neurodevelopmental questions. The *C. elegans* nervous system undergoes extensive rewiring during development.

In particular, *C. elegans* motor neurons increase in number by almost 4-fold following hatch. Extensive rewiring of the motor circuit is required to accommodate these additions, the remodeling of GABAergic dorsal D-class (DD) motor neurons being a particularly striking example. Prior work in the Francis laboratory identified a key role for the homeodomain protein DVE-1 in transcriptional control of DD neuron remodeling. Specifically, *dve-1* is required for the elimination of juvenile synaptic contacts onto DD neurons. Bulk RNA-sequencing (RNA-seq) of *dve-1* mutants identified DVE-1 targets potentially important for synapse elimination. In particular, transcripts encoding the calcineurin-like EF-hand protein CHP1/*chpf-1* and the regulatory subunit of CalciNeurin PPP3R1/*cnb-1* are downregulated in *dve-1* mutants. My studies are focused on investigating functional roles for CHPF-1, CNB-1, and CNP-2 in remodeling. I have found that synapse elimination is delayed in *cnb-1* and *chpf-1* mutants. Interestingly, each candidate shares calcium binding EF hand domains, perhaps implicating their calcium regulatory functions in synapse elimination. In parallel, I am pursuing RNA-seq studies of GABAergic neurons isolated by fluorescence-activated cell sorting (FACS) to identify additional DVE-1 targets important for remodeling. I expect that my findings to date and ongoing experiments will elucidate new molecular mechanisms governing synapse elimination and the remodeling of neural circuits.

1051V **Monoaminergic modulation of intestinal calcium waves** Jeremy T Florman, Mark J AlkemaNeurobiology, Umass Chan Medical School

To understand mechanisms of brain-gut communication, we are studying how amines modulate calcium oscillations in the intestine. To visualize intestinal Ca^{2+} dynamics, we generated an integrated transgenic strain expressing GCaMP6 in the intestine under the *ges-1* promoter. The *C. elegans* intestine generates rhythmic Ca^{2+} waves to drive the defecation cycle. Typically, intestinal Ca^{2+} waves only occur while animals are feeding. However, we found that exposure to the monoamines tyramine and serotonin induced Ca^{2+} dynamics in the intestine in the absence of food. We developed a pipeline to analyze the propagation of Ca^{2+} waves along the length of the body and identified differences in the waves produced by these monoamines. In the absence of food serotonin produced single waves which propagated rhythmically from posterior to anterior, like waves seen when animals are feeding. In contrast, exogenous tyramine produced high-frequency Ca^{2+} ripples that moved both anteriorly and posteriorly between intestinal cells. This indicates a complex role for monoaminergic signaling in intestinal physiology. We found that loss of tyraminergic G-protein coupled receptor TYRA-3 and the tyramine gated chloride channel LGC-55 suppressed the effect of tyramine on intestinal Ca^{2+} dynamics. Previous studies found that repeated activation of the flight response and tyramine release reduces stress resistance through activation of TYRA-3 in the intestine [1]. We hypothesize that tyramine induced calcium transients in the intestine stimulates ILP secretion and the activation of the DAF-2/IIS pathway.

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1052V **Synapse organization and circuit function of dual-transmitter neurons** Andrea Cuentas Condori[1], Patricia Chanabá-López[1], Daniel Colón-Ramos[1,21]Neuroscience, Yale University, [2]Cell Biology, Yale University

A single neuron can use more than one neurotransmitter to signal with neighboring cells and regulate behavior. These dual-transmitter neurons can segregate molecularly distinct presynaptic terminals within the same neuron, but little is known about what are the cellular and molecular strategies that differentially regulate neurotransmitter-specific synaptic pools and how this intracellular specificity shapes circuit function. My work aims to establish an *in vivo* model of dual-transmitter neurons using the well-mapped nervous system of the nematode *Caenorhabditis elegans* to understand how molecularly distinct synapses organize and segregate within a single neuron to regulate animal behavior. To visualize neurotransmitter-specific pools, we have developed tools to monitor the endogenous vesicular transporters of each neurotransmitter, which allow us to systematically validate *in vivo* the dual-transmission capacity of *C. elegans* neurons. Because the *C. elegans* nervous system has several neurons with dual-transmission capacity, the strategies I will develop could be readily implemented to any other neuron and broaden our understanding on how these dual-transmitter neurons assemble within functional circuits.

1053V **One Carbon Metabolism governs efficient neuronal regeneration in *C. elegans*.** Dilip Kumar Yadav, Christopher Gabel Boston Univ Sch Medicine

A damaged and regenerating neuron must reprogram and adapt to allow the production and assembly of cellular components and meet increased energy demands. Recent evidence highlights the critical role of cellular metabolism in this process. We recently discovered that modification of O-linked β- N-acetylglucosamine (O-GlcNAc) signaling can strongly enhance axon regeneration in *C. elegans* through shifts in cellular glycolysis. We have now defined the specific metabolic pathway by which this occurs. Performing *in vivo* laser axotomy and measuring subsequent regeneration of individual neurons in C. elegans, we find that the *ogt-1* mutation increases regeneration by diverting the metabolic flux of enhanced glycolysis towards one carbon metabolism (OCM) and the downstream transsulfuration metabolic pathway (TSP). RNAseq analysis comparing wild-type and *ogt-1(-/-)* supports this finding identifying a large number of differentially expressed genes involved in OCM. We find that the enhance regeneration of ogt-1(-/-) was abrogated by genetic and/or pharmacological disruption of OCM or the serine synthesis

pathway (SSP) linking OCM to glycolysis, but not by disruption of the downstream transmethylation or lipid synthesis pathways. Instead, enhanced regeneration is dependent on acetyl CoA production through downstream cystathionine metabolism via the vitamin B12 independent shunt pathway. Importantly, the beneficial effects of the *ogt-1* mutation can be recapitulated by simple metabolic supplementation of the OCM metabolite methionine. Taken as a whole, our findings unravel the genetic factors and metabolic pathways underlying enhanced regeneration via O-GlcNAc signaling and highlight the therapeutic possibilities of OCM and its related pathways in the treatment of neuronal injury.

1054V **Imaging the breakdown of nervous system dynamics with age and neurodegeneration in *C. elegans*** Gregory Wirak, Andrew Chang, Raghu Nema, Christopher Gabel Physiology and Biophysics, Boston Univ Sch Medicine

In the aging brain, many of the alterations underlying cognitive and behavioral decline remain opaque. Specifically, how normal aging and neurodegeneration change neuronal connectivity and circuit function on the cellular level remains largely unknown. *C. elegans* offers a powerful model for aging research, and with its simple, well-studied nervous system, presents a unique opportunity to measure and understand the system-wide functional alterations in neuronal senescence. Employing volumetric fluorescence microscopy, we have performed multi-neuronal functional imaging across the aged *C. elegans* nervous system. We measure a progressive age-associated breakdown in system-wide organization and temporal continuity that begins in mid-adulthood. Across a population of animals, aging results in a shift in neuronal activity toward higher frequency dynamics and a specific loss of anti-correlated activity (i.e. inhibitory signaling) between neuron pairs. Importantly, the degree of positively correlated activity (i.e. excitatory signaling) remains unchanged resulting in an overall disruption of the systems excitatory/inhibitory balance with age. These effects are recapitulated by mechanisms known to alter GABAergic signaling with increases/decreases in inhibitory GABA signaling partially ameliorating/accelerating the aging effects. However, within a population, aging progression is highly heterogeneous with individual animals declining at varying rates. Measuring behavioral fitness and neuron dynamics of individual animals with age, we are determining the specific aspects of neuron system function that are maintained in healthy aging. Likewise, performing multi-neuron imaging in *C. elegans* models of Alzheimer's Disease, we are determining how neurodegeneration alters the normal aging process. Our work will define the specific changes in neuron activity at the cellular level that cause breakdown in global nervous system dynamics with age and neurodegeneration, enabling a knowledge-based approach to novel neurotherapeutic targets and strategies.

1055V **Discovering the Neuroprotective Role of HSP12.6 in the Dopaminergic Neurons of C. *elegans*** Sarah Shehreen, Oreoluwa Owoseeni, Chukwuanugo Obiaga Biology, Fisk University

Parkinson's disease is a debilitating neurodegenerative disease affecting 1% of the population above 60. Understanding the specific molecules and mechanisms that protect dopaminergic neurons from degeneration and other hallmarks of the disease such as Lewy body formation, amyloid fibrils, and increase in neuromelanin in the substantia nigra, will inform better therapeutic approaches for treatment of Parkinson's patients. HSPB1-CRYAB complex is composed of small heat shock proteins. HSPB1 is found in the neuromelanin granules in the substantia nigra in Parkinson's patients and can prevent the formation of amyloid fibrils due to oxidized dopamine in vitro. Inhibition of CRYAB has been shown to increase clearance of α-synuclein preformed fibrils (PFFs). The C. elegans homolog of this gene, hsp-12.6, is upregulated in the dopaminergic neurons in C. elegans. This leads us to believe this complex plays a necessary and evolutionary conserved role in dopaminergic neurons. Without the presence of HSP-12.6, we expect accelerated neurodegeneration of the dopaminergic neurons because of increased oxidative stress. We are using using fluorescent microscopy to visualize dopaminergic neuronal integrity and oxidative stress over the lifespan of the worm, as well as a survival assay to observe any differences in lifespan between wildtype and hsp-12.6 mutants. We would like to acknowledge NIH R25 Bridges to the Biomedical Doctorate (#2R25GM107754-06A1) and NSF CREST Center for Biological Signatures & Sensing (#HRD2112556) for funding this study.

1056V **Glucose-6-phosphate isomerase isoforms differentially regulate presynaptic glycolysis via genetically-encoded differences in subcellular localization** Ian J Gonzalez[1], Aaron Wolfe[2], Richard Goodman[3], Daniel Colón-Ramos[31]Cell Biology, Yale University, [2]Neuroscience, Yale University, [3]Cell Biology and Neuroscience, Yale University

Glycolytic enzymes are capable of subcellular compartmentalization, yet the mechanisms that underpin their localization, and the role localization plays in regulating glycolysis is poorly understood. We have established an *in vivo* system in the nematode *C. elegans* to examine the neuronal subcellular localization of glucose-6-phosphate isomerase (GPI-1), a glycolytic protein that catalyzes the second step of glycolysis. The *gpi-1* gene encodes two isoforms (GPI-1A and GPI-1B) that differ in 35 amino acids at the N-terminus which is predicted to encode a coiled-coil domain. We observe that the subcellular localization of these two isoforms differ: GPI-1A, which lacks this domain, displays diffuse localization throughout neurites, while GPI-1B displays distinct presynaptic enrichment. This GPI-1B-specific domain is sufficient to drive presynaptic localization of GFP, and mutagenesis of specific residues within the coiled-coil motif eliminates presynaptic enrichment of GPI-1B. To examine the role of this localization in neuronal metabolism, we adapted HYLight, a biosensor for the glycolytic metabolite fructose 1,6 bisphosphate (FBP;

Koberstein et al., 2022), for use in *C. elegans*. We observe differential regulation of FBP levels—and therefore, glycolysis—for these differentially-localized isoforms, with GPI-1B displaying elevated levels of FBP, consistent with elevated rates of glycolysis. We hypothesize that these findings reveal compartment-specific regulation of glycolytic metabolism encoded by differences in subcellular localization of protein isoforms. These studies uncover novel mechanisms of subcellular glycolysis protein localization and allow us to further explore the physiological consequences of this localization on metabolic regulation and physiological function in an intact, *in vivo* system.

1057V **RPM-1 promotes neurodegeneration in a humanized worm tauopathy model** Melissa Borgen, Xinxing Ding BCES, Florida Institute of Technology

The PHR protein RPM-1 is key regulators of neural development, including axon termination and synaptogenesis. Recent studies have also shown post-development roles for the PHR proteins in axon degeneration following injury. Recently, RPM-1 was shown to promote synapse maintenance through genetic interactions with the microtubule stabilizing protein ATAT-2. Synapse loss is an early hallmark of neurodegenerative diseases. In the context of axon termination, *rpm-1* was shown to be genetically negatively regulated by *ptl-1/Tau*. Tau is well known to form protein aggregates in Alzheimer's Disease and Frontotemporal Dementia. These intriguing observations raise an intriguing idea: is RPM-1 involved in Tau-related neurodegeneration? Here we show that loss of *rpm-1* is protective against neurodegeneration in a humanized worm tauopathy model for Frontotemporal Dementia. Using genetics, we tested known members of RPM-1 signaling, *glo-4*, *dlk-1*, *atat-2*, and *ptrn-1* to determine through which pathway RPM-1 could be functioning. Though RPM-1 interacts with several pathways, we show that RPM-1 largely functions in degeneration through its ubiquitin ligase activity. The ubiquitination target of RPM-1, DLK-1, is also shown to be a major driver of the protective effect against tauopathy-related degeneration. These results suggest that RPM-1 signaling is an important component of tauopathy-related neuronal death, which could provide new targets for diagnostics and therapies.

1058V **A circuit for gait selection in *C. elegans* 3D locomotion** Omer Yuval[1], Lukas Deutz[1], Yongxing Wang[1], Thomas P. Ilett[1], Thomas Ranner[1], Netta Cohen[21]School of Computing, University of Leeds, [2]University of Leeds

To move about in their environments, animals constantly need to process information and select the appropriate motor action. In *C. elegans*, sensorimotor processing is often described as a multi-step process culminating in commands issued by pre-motor interneurons to select and modulate gaits by controlling downstream motor circuits. Here we consider novel gait selection observed in 3D fluids.

We begin by describing novel locomotion gaits observed in low and intermediate viscosity 3D fluids which we dub coiling. During coiling, the neck twists around rhythmically either in the clockwise or counter-clockwise directions. The rest of the body responds to form slightly helical body postures. The cyclic head and neck motion, combined with planar (or near-planar) bending waves that propagate along the body, produce rapid rolling of the primary plane of the animal, with opposite chirality to the body posture. Here we present a detailed description of this gait, as well as a 3D biomechanical model that accounts for the key phenomenon.

C. elegans exhibits both clockwise and counter-clockwise coiling behaviours, often alternating between them. Coiling is also exhibited both in the forward and backward directions. We therefore asked what neural circuit can explain the rhythmic rotation of the neck region, and how the chirality of the behaviour is selected. We present a computational model of the RMD circuit, grounded in electrophysiological recordings, and use it to demonstrate either left-right or dorso-ventral undulations. When coupled to SMD neurons, we show that fictive coiling can be obtained, with either chirality. We confirm the finding by testing corresponding patterns of activation in our biomechanical simulation framework. We then asked what circuit might be responsible for gait selection corresponding to the chirality of coiling undulations. Using our computational framework, we show that a brief pulse applied to a single RMD neuron can induce a switch in the chirality of the fictive pattern. Experimental predictions are presented. Unlike gait selection by interneurons (e.g. AVA and AVB that control downstream motor circuits), we propose that a single motor circuit is tasked both with the generation and selection of chiral gaits.

1059V **Using *C. elegans* to study chemotherapy-induced peripheral neuropathy (CIPN)** Aleksandra Chudinova[1], Lingxin B Wang[1,2], Chandni B Jaisinghani[1], Miriam B Goodman[11]Molecular & Cellular Physiology, Stanford University, [2]GSK

More than half of adult and 100% of pediatric cancer survivors treated with the chemotherapeutic vincristine experience CIPN [1]. This condition manifests as numbness, tingling, muscle weakness, and 30% of people with CIPN also experience chronic pain [1]. The intensity of symptoms and the degree of skin denervation are dose dependent. Severe CIPN leads clinicians to reduce doses of vincristine, which compromises patients' survival. Moreover, in more than 30% of pediatric cancer survivors, CIPN persists years after completion of therapy and can lead to significant sensorimotor impairments throughout life [1]. There is no FDA-approved drug to prevent or alleviate CIPN [2] and the molecular basis of this condition remains poorly understood.

To address this challenge, we are using the well-studied touch receptor neurons in *C. elegans* as a model. Our working hypothesis is that CIPN is caused by a failure of homeostatic regeneration of sensory neurons, leading to degeneration outpacing regeneration. Genetic entry points for this study include the finding that DLK-1 dual-leucine kinase is required for TRN regeneration following laser axotomy [3,4] and that genetic and chemical disruption of microtubules impairs touch sensation in a manner that depends on signaling by DLK-1 and its downstream partners, including PMK-3 p38 MAP kinase. The DLK-1/p38 MAP kinase converges on the CEBP-1 and MEC-3 transcription factors and its activation is seen to reduce expression of MEC-3 and its targets [5], including the MEC-4 ion channel required for touch transduction [6]. A challenge in these studies and in others reliant on treating *C. elegans* with chemicals is the cuticle's permeability barrier. We use *bus-17(br2)* animals to reduce this barrier [7] and although these animals do display a mild touch-insensitivity in the absence of drug treatment. Consistent with the idea that activation of DLK-1 signaling contributes to CIPN, we found that DLK-1(OE) in the TRNs is sufficient to impair touch sensation. Treating *bus-17(br2)* with vincristine impairs touch sensation and this effect is suppressed in *bus-17(br2);pmk-3(ok169)* double mutants. We are investigating both upstream and downstream signaling partners in order to determine their role in vincristine-induced touch defects as well as designing experiments to directly visualize regeneration and its modulation by vincristine.

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1060V **CeNGEN: Goals and Approaches** Marc Hammarlund[1], Oliver Hobert[2], Smita Krishnaswamy[3], David M Miller[41]Genetics and Neuroscience, Yale School of Medicine, [2]Biological Sciences, Columbia University/HHMI, [3]Genetics and Computer Science, Yale School of Medicine, [4]Cell and Developmental Biology, Vanderbilt University

The CeNGEN project (*C. elegans* Neuronal Gene Expression Map & Network) is using RNA sequencing to describe gene expression in individual neuron types. Previously, we cataloged transcripts for every known neuron in the L4 hermaphrodite (Taylor et al., 2021). We are currently working on the following goals:

1. A time-resolved atlas of neuronal gene expression in hermaphrodites. We are using single-cell RNA seq (scRNA-Seq) for gene expression throughout development with the goal of generating a gene expression map that parallels the cell lineage. We will analyze gene expression changes along neuronal lineages and in terminally differentiated neuron types by using computational approaches (MIOflow, Huguet 2022) that infer cellular dynamics from static snapshots.

2. A time-resolved atlas of neuronal gene expression in males. We are using scRNA-Seq to catalog gene expression in male neurons across development. For sex-shared neurons, we are identifying sexually dimorphic transcripts using a manifold density estimation approach (MELD, Burkhardt 2021) that distinguishes male vs hermaphrodite cells. For male-specific neurons, we will describe gene expression in every neuron type.

3. Alternative RNA processing and expression of non-polyadenylated genes across all neuron types. Alternative RNA processing drives neuronal diversity. We are using bulk and sc sequencing to describe alternative splicing in all neuron types in the hermaphrodite. Our approach also captures non-polyadenylated RNAs, many of which show striking, neuron-specific expression. We will train a novel multimodal network consisting of a transformer, a graph neural network and an MLP to create a joint embedding of splice site sequence, structure, and contextual information, from which we will predict alternative splicing. The neural network will feature attention mechanisms for interpretability.

4. Computational and machine learning approaches to analyze expression data and deploy web-accessible tools for data mining. We continue to update CeNGENApp, which has become a widely-used resource for the scientific community. We are developing novel computational approaches that will power analysis of gene expression in individual neuron types across development and sex.

The completion of this work will provide molecular insight into gene expression in the male and hermaphrodite nervous systems and address how neuronal diversity is generated and maintained.

1061V **It is time to do the experiments needed to reverse-engineer the *C. elegans* nervous system** Gal Haspel[1], Edward S. Boyden[2,3], Konrad P Kording[41]Biological Sciences, New Jersey Institute of Technology, [2]Departments of Brain and Cognitive Sciences, Media Arts and Sciences, and Biological Engineering, Massachusetts Institute of Technology, [3]McGovern Institute and HHMI, [4]Department of Bioengineering and Department of Neuroscience, University of Pennsylvania

The problem: The key goal of much of systems neuroscience is to describe and understand the building blocks that compose nervous systems and how these building blocks work collaboratively to become more than the sum of their parts, enabling the impressive behaviors of animals. This process of reverse engineering should produce a simulation that replicates the be-

havior repertoire and predicts all activities and the effects of any perturbation. Arguably the key building block is the neuronal Input-Output Function (IOF), i.e. the function that characterizes how a neuron integrates its inputs and responds to them, and characterizes the local computation that each neuron performs. Ultimately, the interplay of all the neurons' IOFs gives rise to neural dynamics and behavior. And yet, there is no complete measurement of IOFs in any animal. We propose to do just this in the model system *C. elegans*, developing methods and discovering principles that will generalize beyond.

Scalable approaches: Because neuronal IOFs likely include nonlinear interaction of inputs, this effort requires recording from most or all neurons while stimulating at least all pairs of neurons, requiring many experiments, in the order of at least 10,000. With microfluidics, optogenetics, and modern microscopy, it is possible to target individual neurons and subsets of neurons in the nematode while recording identifiable neuronal activity across the nervous system (via calcium or voltage indicators). We propose new genetically-encoded constructs and microscopy hardware, all optimized and validated specifically for this project, that will enable the parallel collection of the required data on multiple setups. Finally, a portion of the animals will be collected for expansion microscopy, enabling the mapping of transcriptomic gene expression patterns and key proteins with nanoscale precision, providing individual molecularly annotated connectomes of the very animals that underwent live-imaging experiments. Large-scale fitting then reveals the IOFs.

A commitment to open science: All our data will be shared the day we acquire it. All our code will be shared while we develop it. We will encourage the community to collaborate with us in experimental design, technology development, and computational and theoretical analysis.

1062A **Preliminary RNAi screen for genes involved in maintenance of youthful behavior** Rex A Kerr, Cynthia Kenyon Calico Life Sciences LLC

The biological basis of aging is of widespread importance, both because of its relevance to society, and because it is practically ubiquitous in living organisms yet not well-understood in mechanistic detail. In particular, relatively little is known about which genes are involved in the maintenance of or accelerated decline from youthful behavior, especially in the case where the gene may not have a substantial impact on longevity.

In order to better characterize the genetic basis of the maintenance of youthful behavior, we are conducting an exploratory RNAi screen using the *C. elegans* Observatory, a high-throughput behavioral analysis platform that we have developed. Previous experiments on sets of candidate genes have confirmed behavioral impacts of known longevity genes, including *daf-2* and *hsf-1*, and revealed previously unknown age-related behavioral phenotypes in genes not typically implicated in *C. elegans* longevity, including *tank-1* and *cdc-42*.

We therefore are conducting an unbiased pilot RNAi knockdown screen to assess whether genes affecting behavioral aging are common or uncommon in the genome. Because we have characterized the behavioral variability from the Observatory, and thereby have strong predictions for our chance of detecting effects of various sizes, both positive and negative results can be interpreted. We will provide an update on our latest results, plus share the results from small candidate gene screens run concurrently with the unbiased screen.

1063A **Robotic device for fully automated high-content screening on *C. elegans* as a novel NAMs platform for chemical toxicity assessment** Laurent Mouchiroud, Elena Katsyuba, Morgane Bourgeois, Lazar Stojkovic, Fabien Tâche, Matteo CornagliaNagi Bioscience SA

Nematode *Caenorhabditis elegans* constitutes a valuable NAMs model for multiple applications, including predictive toxicology. This microscopic worm gained popularity for its ideal short size and life cycle, ease of cultivation and propagation, and powerful genetic toolkit. While *C. elegans* has the potential to complement *in vitro* models to better predict toxic outcomes in mammals, the current experimentation methods lack automation and standardization, limiting their wider use in screenings.

In response, we developed a microfluidic-based robotic platform that automates the entire process of *C. elegans* culture, treatment, high-content imaging, and phenotypic analysis. The platform is able to execute multiple toxicity assays, including the possibility of using the existing ample collection of reporter strains thanks to the fluorescent imaging capability.

As an illustration, we evaluated the reproductive and developmental effects of twenty benchmark chemicals on *C. elegans* using the proposed platform. Synchronized populations of worms were chronically exposed to five doses of test compounds starting from the last larval stage (L4). Time-resolved phenotypic readouts were automatically extracted from the hourly-collected images of the worms, including growth dynamics, sexual maturity, fertility, embryonic viability, progeny accumulation and survival rate. Out of the tested compounds, methotrexate showed the most pronounced embryonic viability adverse effects, while bisphenol A strongly impacted the mothers' development.

Overall, we propose an innovative solution for rapid identification of toxic compounds and their mechanism of toxicity, bridging the gap between *in vitro* and *in vivo* assays. Our technology allows not only endpoint measurements' collection, but also the monitoring of biological responses' dynamics.

1064A **A robotic platform for fully automated ageing studies in *C. elegans*** Elena Katsyuba, Morgane Bourgeois, Lazar Stojkovic, Fabien Tâche, Matteo Cornaglia, Laurent Mouchiroud Nagi Bioscience SA

C. elegans is a powerful model organism for ageing studies. However, the traditional protocols, which continue to be broadly used, rely on manual handling, making them labor-intensive and time-consuming. Automation of these processes would greatly benefit long-term studies of *C. elegans*. Significant progress has been achieved over the past decade in the techniques to study worm's biology: the introduction of microfluidic approaches for different assay types and the use of machine learning-based algorithms for data processing offer an increase in experimental throughput and a better control of experimental conditions.

We propose here a novel solution for automated ageing studies in *C. elegans*, which involves these new methodologies. Our microfluidic-based robotic platform is capable to fully automate all the key aspects of *C. elegans* experimentation, including worm culture, treatment, imaging, as well as data recording and analysis. The unique characteristics of the platform allow ageing studies on multiple worm populations in parallel that go beyond a simple tracing of the survival curves. We present here a panel of standardized bioassays allowing automated: (1) monitoring of *C. elegans* lifespan, (2) assessment of worm fitness, (3) testing of different stress responses activation, (4) identification of developmental phenotypes that can serve as potential predictors of ageing.

The performance of the assays was corroborated by testing benchmark compounds known to affect C. elegans longevity, as well as different dietary regimens. While the results obtained with our platform were consistent with the results obtained with conventional "manual" methods on plates, the use of microfluidic chips significantly reduced the consumption of test compounds, and fully automated imaging process and data analysis software significantly reduced the number of man-hours required for such study.

1065A **Rescuing synaptic defects in mitochondrial Complex I deficiency models with natural compounds** Silvia Maglioni[1,2], Alfonso Schiavi[3], Marlen Melcher[4], Vanessa Brinkmann[3], Zhongrui Luo[5], Jiwon Jung[3], Anna Laromaine[5], Nuno Raimundo[6], Joel N Meyer[7], Felix Distelmaier[4], Natascia Ventura[2,31]IUF Leibniz Research Institute EnvironmentalMed, [2]Institute for Clinical Chemistry and Laboratory Diagnostic, Medical Faculty of the Heinrich Heine University, [3]IUF-Leibniz Institute for Environmental Medicine, [4]Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Heinrich-Heine-University Düsseldorf, [5]Institut de Ciència de Materials de Barcelona, ICMAB-CSIC. Campus UAB, [6]Department of Cellular and Molecular Physiology, Penn State College of Medicine, [7]Nicholas School of the Environment, Duke University

Human mitochondria-associated diseases (HMAD) are clinically heterogeneous and multifaceted disorders. Among those, Complex-I (CI) deficiency represents the most frequently observed. Unfortunately, therapeutic options for these devastating disorders, which in most cases present with neurodevelopmental defects, do not exist, in part due to the absence of appropriate model systems to study them.

We used our recently generated *C. elegans* models for mitochondrial CI deficiency to carry out a screening in search for disease suppressors. Specifically, our models exploited RNA interference to silence *nuo-5*/NDUFS1 gene (as well as other CI subunits) in the nematode, which lead to neurometabolic deficits and arrested animals' development.

Our suppressor screening identified lutein, one of the most common carotenoids found in the human diet and body tissues (and able to pass the blood brain barrier), for its ability to rescue the developmental arrest and neuronal deficits observed upon *nuo-5* depletion. We specifically found that lutein exerts its beneficial effects by rescuing a neuroligin(*nlg-1*)-mediated synaptic defect we disclosed for the first time upon *nuo-5* depletion (Maglioni et al. *Nat Commun* 2022).

Our ongoing research involves testing the efficacy of lutein, both alone and in combination with other candidate compounds identified in our screen, on other CI-disease models, as well as in new *nuo-5*/NDUFS1 knock-in models that we recently generated in our laboratory. Our preliminary data show the effectiveness of lutein (and kahalalide F) in rescuing neuromuscular defects in other CI genes-depleted animals (namely *nuo-1*, *lpd-5*, F53F4.10, and T20H4.5) as well as in the genetic mutant.

With this work we aim at establishing new nematodes models to study HMAD and at the same time to identify new potential therapeutics for this class of diseases which urgently needs effective treatment options.

1066A **Age-dependent nuclear lipid droplet accumulation is a cellular hallmark of ageing** Christina Ploumi[1], Meropi Mari[2], Geroge Filippidis[2], Nektarios Tavernarakis[3], Konstantinos Palikaras[11]Department of Physiology, National and Kapodistrian University of Athens, [2]Institute of Electronic Structure and Laser, Foundation for Research and Technology, [3]Institute of Molecular

Lipid droplets have been shown to localize in most nuclear compartments, where they impinge on genome architecture and integrity. However, the significance of progressive nuclear lipid accumulation and its impact on nuclear morphology and organismal homeostasis remain obscure. Here, we implement non-linear imaging modalities to monitor and quantify age-dependent nuclear lipid deposition. We find that lipid droplets increasingly accumulate in the nuclear envelope, during ageing. Longevity-promoting interventions, such as low insulin signaling and caloric restriction, abolish the rate of nuclear lipid accrual and decrease the size of lipid droplets. Suppression of lipotoxic lipid accumulation in intestinal nuclei is dependent on the transcription factor HLH-30/TFEB and the triglyceride lipase ATGL-1. HLH-30 regulates the expression of ATGL-1 to reduce nuclear lipid droplet abundance in response to lifespan-extending conditions. Notably, ATGL-1 localizes to the nuclear envelope and moderates lipid content in long-lived mutant nematodes during ageing. Our findings indicate that the reduced ATGL-1 activity leads to excessive nuclear lipid accumulation, perturbing nuclear homeostasis and undermining organismal physiology, during ageing.

1067A **Lactate promotes longevity via remodeling of metabolic signals in *C. elegans.*** Arnaud Tauffenberger[1], Hubert Fiumelli[2], Frank C. Schroeder[1], Pierre Magistretti[21]Boyce Thompson Institute, [2]KAUST

Aging is a primary risk factor for neurological disorders during middle to late adulthood. A common feature of aging and neurological disorders is a reduction in the efficiency of energy metabolism, and in particular the maintenance of intracellular homeostasis. Aging is a plastic process, which can be ameliorated by genetic, dietary, and pharmacological interventions. L-lactate has been associated with multiple cellular functions, ranging from reducing inflammation, improving muscle biogenesis and synaptic plasticity. Additionally, L-lactate can improve resilience to stress and extend longevity in *C. elegans*. The molecular mechanisms underlying lactate-dependent extension of longevity remain to be identified. In this study, we analyzed lactate-induced changes in transcriptome to identify alterations in the transcriptional network underlying aging. For this purpose, we profiled *C. elegans* at multiple timepoints during adulthood. We observed that lactate supplementation is associated with a remodeling of the transcriptional landscape at mid-adult stages and with little impact during development. Lactate induced expression of genes within signaling cascades associated with cellular homeostasis (proteasome, redox changes, detoxification mechanisms) while metabolic processes (mitochondrial respiration, lipid metabolism) were strikingly reduced. In parallel, an untargeted metabolomic analysis revealed that lactate can regulate modular glucosides (MOGLs) that accumulate during aging. Lactate was able to limit the accumulation of these age-associated small-molecules, opening new ways to investigate how metabolic changes influence aging. This multi-omics approach will contribute to elucidate how lactate impacts cellular physiology and homeostasis. This could unveil new targets to be validated in higher organisms regarding their function in cell survival, especially in the context of neurological disorders.

1068A **Genetic dissection of NHR-49-driven stress resistance pathways** Kelsie RS Doering[1,2], Glafira Ermakova[1], Stefan Taubert[11]The University of British Columbia, [2]Kwantlen Polytechnic University

The pathways that regulate a cell's and animal's response to many stresses are highly evolutionarily conserved. Master regulators of gene expression are often required to respond to and survive these stresses, including oxidative stress, hypoxia, and starvation, which require SKN- 1/Nrf2, HIF, and HLH-30/TFEB, respectively. Our lab has shown that the nuclear hormone receptor NHR-49 is a key component of the regulatory response to these stresses. To uncover novel factors acting with NHR-49 to regulate responses to tBOOH, hypoxia, and starvation, we conducted a large-scale reverse genetic screen, revealing a set of 37 genes which may act within the NHR-49 regulated responses to all three stresses. The protein kinase *hpk-1* is required for worm survival to all three stresses, but only acts in the same pathway as *nhr-49* in the hypoxia and oxidative stress response. The pseudokinase *nipi-3* is required together with *nhr-49* for hypoxia survival, shows resistance to tBOOH, but is not required for starvation survival. In addition, the nuclear receptor *nhr-80*, a dimerization partner of NHR-49 for the regulation of fatty acid desaturation and life span regulation, acts in the same pathway as *nhr-49* or survival to hypoxia and oxidative stress, but is not required for starvation survival. This work has uncovered pertinent factors that may delineate stress-specific or general signaling branches of the NHR-49 stress response pathway.

1069A ***mul-1* integrates DNA damage and oxidative stress responses in *C. elegans*** Emilio Carranza-Garcia, Anton GartnerGenetic and Genomic Toxicology Branch, Center for Genome Integrity, Institute for Basic Science

DNA damage responses occur at the cellular level, but also involve organismal responses and cross-talk with other stress pathways. *Caenorhabditis elegans* provides an ideal system to study the integration of pathways that are activated in response to ionizing radiation (IR). IR activates the *pmk-1*/p38 MAP kinase pathway and the downstream transcription factor *ATF-7*/ATF2/ATF7/CREB in gut cells, which results in the activation of the core apoptotic machinery in the germ line to induce apoptosis [1]. Previous work by our lab reveals the upregulation of 41 genes in IR-treated animals and only three are dependent on *cep-1*/p53. Most of the upregulated genes are related to the response to oxidative stress, innate immunity and ageing [2]. F49F1.6,

also termed as *mul-1* (MUcin Like gene) shows homology with the human mucin MUC2 and is predicted to encode a secreted surface protein. Interestingly, our previous genome-wide gene expression analyses indicate that *mul-1* is increased 155-fold 2 h after treating animals with IR (120 Gy) [2]. *mul-1* is also involved in the cross-tolerance between IR and pathogen infection [3].

Here, we utilized CRISPR-Cas9 genome editing to generate transcriptional and translational reporters to analyze the organismal response to IR and other stressors. Our results show that IR induces a transcriptional upregulation of *mul-1* in gut cells. This induction is also achieved under oxidant conditions (H_2O_2), but not under heat shock. We demonstrate that *mul-1* requires the *pmk-1*/p38 pathway upon both, IR and H_2O_2 treatment. We also show evidence that suggests a role for *mul-1* in DNA damage-induced germ cell apoptosis by protecting germ cells from death after IR exposure. These findings suggest an interplay between DNA damage response pathways and pathways related oxidative stress.

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1070A **Sleep deprivation exacerbates amyloid beta aggregation** Priscila Yumi Tanaka Shibao[1], Philip Zimmer[2], Henrik Bringmann[3], Janine Kirstein[1,41]Department of Cell Biology, University of Bremen, [2]University of Bremen, [3]Biotechnology Center, Technical University Dresden, [4]Leibniz Institute on Aging, Fritz Lipmann Institute

Alzheimer's Disease (AD) is the major cause of dementia and is estimated to affect more than 150 million people by 2050. The hallmarks of AD are the accumulation of extracellular amyloid plaques and intracellular tau tangles. Clinical data suggests that cognitive decline and changes in the pattern and quality of sleep are correlated in non-demented people, and this correlation is stronger in the demented elderly. In addition, only one night of sleep deprivation (SD) can lead to an increase in the level of amyloid beta$_{1-42}$ (Aβ) in the blood of AD patients. This poses the question of how SD can affect Aβ aggregation. To address this question, we employed the AD *C. elegans* model established in our lab (JKM2), in which the human Aβ$_{1-42}$ is expressed under the control of the pan-neuronal *rgef-1* promoter and sub-stoichiometrically labeled with mScarlet (nAβ). The control strain (JKM3) expresses only mScarlet pan-neuronally (nScarlet). Our models enable the measurement of Aß aggregation by fluorescence lifetime imaging (FLIM). We crossed the AD and control strains with a SD *C. elegans* strain (HBR227) which is mutated in the *aptf-1* gene. FLIM analysis revealed a significant increase of Aβ aggregation in all examined head neurons in L4 and day 6 old SD nematodes. In addition, the SD AD nematodes were further impaired in their organismal fitness and produced less viable offspring. The results suggest that SD exacerbates Aβ aggregation and its associated proteotoxicity. We are currently analyzing the molecular and neuronal circuits on how SD advances amyloid aggregation.

1071A **A dicer-related helicase opposes the age-related pathology from SKN-1 activation in ASI neurons** Chris D Turner[1], Nicole L Stuhr[1], Carmen M Ramos[1], Bennett T Van Camp[1], Sean P Curran[21]Molecular Biology, University of Southern California, [2]Leonard Davis School of Gerontology, University of Southern California

Coordination of cellular responses to stress are essential for health across the lifespan. The transcription factor SKN-1 is an essential homeostat that mediates the response to environmental stress conditions and cellular dysfunction, but constitutive activation of SKN-1 drives premature aging thus revealing the importance of turning off this cytoprotective response. Here we identify how SKN-1 activation in two ciliated ASI neurons in *C. elegans* results in an overload in organismal transcriptional capacity that drives pleiotropic outcomes in peripheral tissues. An increase in the expression of established SKN-1 stress response and lipid metabolism gene classes of RNA in the ASI neurons, in addition to the increased expression of several classes of non-coding RNA, define a molecular signature of animals with constitutive SKN-1 activation and diminished healthspan. We reveal neddylation as a mediator of SKN-1 within intestinal cells. Moreover, RNAi-independent activity of the dicer-related DExD/H-box helicase, *drh-1*, in the intestine, can oppose the effects of aberrant SKN-1 transcriptional activation and delays age-dependent decline in health. Taken together, our results uncover a cell non-autonomous circuit to maintain organism-level homeostasis in response to excessive SKN-1 transcriptional activity in the sensory nervous system.

1072A **Crosstalk between viral dynamics and host responses in the *C. elegans*-Orsay virus pathosystem** María Victoria García Castiglioni[1], Maria J Olmo-Uceda[1], Ana Villena[1], Juan C Muñoz-Sánchez[1], Santiago F Elena[1,21]Institute for Integrative Systems Biology, [2]Santa Fe Institute

Orsay virus (OrV) is the only identified natural virus of *C. elegans*. OrV follows an oral-fecal transmission and replicates inside intestinal cells. Infection has very little impact on the worm's fitness, indicating that the activation of the innate immunity is efficient against the virus. Here we aimed to shed light into the interactions between *C. elegans* and OrV by following the course of an infection during development. For this purpose, we studied both the dynamics of virus accumulation and the responses elicited in the worm.

By examining the progression of the viral load we observed that infection could be divided into four stages: (1) pre-infection, (2) exponential viral replication leading to a viral load peak, (3) host-pathogen conflict leading to a second lower viral load peak, and (4) persistent residual infection. We observed that the ratio of the two genomic RNAs varied during the different phases of the infection, with a higher production of the RNA2 – which encodes the capsid proteins – coinciding with the peaks in viral load. Relatedly, we started seeing signs of viral egression after the first peak, suggesting that a viral generation would be complete. Remarkably, the number of infected cells and the relative infected area remained mostly stable along infection.

In order to investigate the response elicited in the worm, we looked at the changes in the transcriptome, along the four infection stages. As a result, we obtained a temporal transcriptomic landscape of the genes involved in the response to infection. Analysis of genes that change similarly across time identified different groups, including one cluster of genes with a similar profile to the viral load and other clusters enriched in metabolic processes which may be relevant to the viral cycle. We also saw responses to OrV that are frequently linked to other biotic stressors, suggesting that the immune response triggered by OrV may be more general than previously thought. Moreover, we found certain genes involved in development among the differently expressed genes, suggesting that the virus may be desynchronizing the worm populations. Finally, we identified early responding genes and analyzed the progression of the viral load in mutants of these genes, pinpointing new players involved in the viral response to OrV.

1073A **Is there an advantage to polyploidy in animals? Consequences of whole genome duplication in a synthetic *C. elegans* tetraploid** Laetitia Chauve, Liam Butler, Aoibhin McGarry, Aoife Mc Lysaght Genetics Department, Trinity College Dublin

Gene duplications play a major evolutionary role by providing raw material for functional innovation. **Whole Genome Duplication (WGD)**, or polyploidization, is a particular case of duplication, where the entire genetic sequence is repeated within the nucleus. In plants, WGD is recognized as a major evolutionary force, and is linked to speciation and the ability to resist periods of stress and of environmental upheaval. In animals, examples of current polyploid species are rarer, but we know of several ancient events of WGD: for instance, two rounds of WGD occurred during early vertebrate evolution. Those events are usually followed by gene loss and diploidization, processes which reshape the genome and channel evolutionary outcomes. The reason for the success of polyploidy in animals is unclear. Stressful conditions (heat or cold) can generate WGD and one debated hypothesis states that polyploidy is adaptive on the short-term, however this has never been studied in animals. This question is also relevant for cancer, as polyploidy is strongly correlated with drug resistance and poor prognosis in several types of cancers.

Our goal is to understand the physiological consequences of polyploidy and its potential adaptive consequences. We are investigating the **consequences of polyploidy in *Caenorhabditis elegans***, where tetraploidy can be artificially generated by transient knock-down of *rec-8*, a cohesion component complex (Clarke *et al*, *JoVE,* 2018). We have characterized the consequences of tetraploidy on *C. elegans* physiology and stress resistance. Our results reveal that although tetraploidy reduces fitness by decreasing fertility and lifespan in regular conditions, tetraploid animals exhibit **increased resistance under specific stress conditions, related to temperature changes**. While tetraploids exhibit similar pathogen resistance, their response to heat stress is altered. They exhibit slightly improved thermotolerance and prolonged *hsp* (heat shock protein) mRNA induction upon heat shock (HS). These phenotypes can be linked with altered *hsp-16.2* nuclear localization upon HS, using an in-vivo tractable system (Rohner *et al*.,*J. Cell Biol*, 2013). Furthermore, tetraploids exhibit dramatic increase in recovery from severe cold stress at the adult stage. These results might help explain recent reports showing correlation between the frequency of extant animal polyploids and higher latitude regions, recent glaciation, and large temperature variation (David, *PNAS,* 2022).

1074A **Non-vesicular transport of cholesterol in XXX cells regulates dauer formation in *C. elegans*** Raihanah Harion, Jingbo Sun, Yasunori SahekiLee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Adaptation to environmental stresses through phenotypic plasticity is an important response for organismal survival and reproductive development. *C. elegans* responds to adverse conditions such as nutrient deprivation by undergoing the stress-resistant dauer process. A key step in this process is the synthesis of cholesterol-derived steroid hormones, namely dafachronic acids (DAs), which occurs in the endoplasmic reticulum (ER) within specific cell types, including the neuroendocrine XXX cells. As *C. elegans* cannot synthesize cholesterol, it relies on external supply of cholesterol for DA synthesis. However, how cholesterol reaches the ER to support the DA synthesis remains unclear. Here we show that the *C. elegans* homolog of GRAMD1/Aster family of cholesterol transporters (ZC328.3) plays an important role in regulating dauer formation via its cholesterol transporting function in XXX cells. Upon increase in cholesterol levels in the external environment, ZC328.3 becomes highly expressed in these

cells. Animals that lack ZC328.3 together with NCR-1/NCR-2 [*C. elegans* homologs of Niemann-Pick type C 1 (NPC1) involved in cholesterol egress from lysosomes] exhibited much enhanced dauer formation compared to animals that lack NCR-1 and NCR-2 alone. Importantly, the formation of dauer was completely suppressed by supplementing DAs to these animals. ZC328.3 localizes to the close proximity of lysosomes, suggesting its potential role in transporting cholesterol from lysosomes to the ER to promote the biosynthesis of DAs. Our studies provide important insights into the molecular basis of cholesterol transport to the ER and its impact on adaptation to various environmental stresses.

1075A **Unravelling effects of anti-aging drugs on *C. elegans* using liposomes** Aihan Zhang, Carina Carla Kern Genetics, Evolution and Environment, University College of London

There is growing interest in the development of anti-aging drugs and *C. elegans,* in principle, seems an ideal model organism to screen for them in. This is reflected by the recent establishment of several biotech companies that employ the worm in this way. However, such screens remain subject to technical limitations, such as complex interactions between drugs and the worms' bacterial food source, and failure of drugs to be taken up into worm tissues. We have explored the use of liposomes to overcome these shortcomings [1]. Liposomes improved drug uptake, particularly because they concentrate compounds at the surface of bacterial lawns, increasing bioavailability. This also greatly reduces (~200-fold) the amount of drug needed, which lowers drug costs. However, liposomes do not guarantee uptake of any compound into worm tissues.

We also used liposomes to examine the role of drug-*E. coli* interactions in effects of compounds previously reported to increase lifespan. Trimethadione and glutathione extended lifespan only if delivered using liposomes. Thioflavin T (ThT) and glutathione only extended lifespan in the presence of proliferating *E. coli.* Here, glutathione acts by increasing worms' infection resistance, possibly via an innate immune training effect [2]. By contrast, ThT extends lifespan largely by suppressing bacterial infection due to its antibiotic properties (c.f. carbenicillin). Notably, for rapamycin, preventing bacterial proliferation revealed a larger life-extending effect.

Together, these findings provide a toolbox of information to help strengthen the capability of future studies using *C. elegans* to reliably identify anti-aging drugs.

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1076A **Investigating kynurenine-pathway mediated neuroprotection in ageing** Anna Ainslie, Renée Seinstra, Yifan Wang, Martijn van Faassen, Claude van der Ley, Ido Kema, Ellen NollenUniversity Medical Center Groningen

With a progressively ageing population, the need to understand mechanisms of neuroprotection is increasing. It is well established that depletion of the Kynurenine Pathway (KP) enzyme tryptophan 2,3-dioxygenase (TDO-2) rescues motility and lifespan in ageing worms and in multiple neurodegenerative disease models. However, our understanding of the precise mechanisms and tissue-specificity of neuroprotection by TDO-2 depletion is still limited. In our research, we use the *C. elegans* model to elucidate the dynamic local and systemic changes that are induced by TDO-2 depletion. We have optimized a novel technique (split-wrmScarlet) for tissue-specific visualization of TDO-2 and other KP enzymes, in order to analyze the precise tissue-localization and dynamics in ageing. Preliminary analysis suggests that TDO-2 is primarily expressed in the hypodermis throughout adulthood, an organ that transcriptomically mimics the human liver in *C. elegans*. Additionally, we have established a pipeline to perform tissue-specific depletion (using auxin-inducible degradation) of TDO-2 and other KP enzymes, and subsequently analyze dynamic changes in KP metabolite levels and motility over time. Overall, this data will help us understand the tissue-specific role of KP enzymes and metabolites in neuroprotection, and thus elucidate potential mechanisms to help fight neurodegeneration in ageing.

1077A **An AI apporach to quantifying *Nematocida parisii* infection within *C. elegans*** Edward James, Aaron W ReinkeMolecular Genetics, University of Toronto

Microsporidia are fungal pathogens which infect most animal species. *C. elegans* is naturally infected by multiple species of microsporidia, with *Nematocida parisii* being the most common and the best studied. *N. parisii* infects and reproduces within intestinal cells, negatively impacting *C. elegans* development, reproductive fitness, and survival. Infected *C. elegans* can be visualized by staining spores with the chitin-binding dye DY96. This dye also stains *C. elegans* embryos, which can be counted to determine the relative impact of infection on the host. Analyzing data containing DY96 stained microsporidia involves manual outlining of each *C. elegans,* and manual thresholding of the DY96 channel to remove embryos leaving just the microsporidia signal; a time-intensive and prohibitive bottleneck to high-throughput experiments. To adapt the *C. elegans*/microsporidia system for high-throughput experimentation, we developed a machine learning tool to identify *C. elegans,* embryos, and microsporidia in multi-channel microscope images. *C. elegans* are identified from a DAPI channel via a custom YOLOv8 instance segmentation network. The DY96 channel for each identified *C. elegans* is then interrogated for embryos via a custom YOLOv8 object

detection network, and for microsporidia via a pixel-level random forest classifier, making DY96 a pseudo-specific microsporidia probe. Our AI pipeline allows automated measurement of both *C. elegans* and microsporidia fitness in response to experimental manipulation, directly measuring replication via embryos and spores. Being able to automatically quantify host and microsporidia fitness in combination will enable high-throughput experiments such as RNAi and drug screens. While the random forest classifier is specific to DY96/microsporidia, our approach could be adapted to other *C. elegans*/pathogen systems.

1078A **Bacterial expression cloning reveals *C. elegans* and tardigrade proteins that can protect drying cells** Jon Hibshman[1], Courtney Clark-Hachtel[1], Bob Goldstein[1,21]Biology Department, The University of North Carolina at Chapel Hill, [2]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill

Dehydration causes extensive damage to cells, yet some animals are able to survive extreme desiccation. The protectants and molecular mechanisms that facilitate desiccation tolerance remain largely unknown. We devised an expression cloning approach to screen for novel protein protectants from desiccation-tolerant animals including *C. elegans* and tardigrades. We expressed cDNA libraries in *E. coli* and subjected the bacteria to desiccation. Population sequencing revealed that tardigrade mitochondrial single-stranded DNA-binding proteins (mtSSBs) were enriched among the expression libraries from bacteria that survived desiccation. Individual expression of each mtSSB in bacteria validated them as potent desicco-protectants in bacteria. The DNA-binding domain of the protein was both necessary and sufficient for improving bacterial desiccation tolerance, and mutations of predicted DNA-binding amino acids reduced the ability of these proteins to act as desicco-protectants. From expression cloning screens in *C. elegans*, we identified many genes that were enriched following desiccation, several of which we have validated as desicco-protectants. Interestingly, some of these are also DNA-binding proteins. The ability of tardigrade mtSSBs and other worm DNA binding proteins to promote desiccation survival in bacteria suggests that DNA binding may be a common means of desicco-protection. This work demonstrates that expression cloning is an efficient method to discover novel protectants and has identified proteins from tardigrades and *C. elegans* that can function as potent desicco-protectants.

1079A **LEA motifs promote desiccation tolerance in vivo** Jon Hibshman[1], Bob Goldstein[1,21]Biology Department, The University of North Carolina at Chapel Hill, [2]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill

Cells and organisms typically cannot survive in the absence of water. However, some animals including nematodes, tardigrades, rotifers, and some arthropods are able to survive near-complete desiccation. One class of proteins known to play a role in desiccation tolerance is the late embryogenesis abundant (LEA) proteins. These largely disordered proteins protect plants and animals from desiccation. A multitude of studies have characterized stress-protective capabilities of LEA proteins in vitro and in heterologous systems. However, the extent to which LEA proteins exhibit such functions in vivo, in their native contexts in animals, is unclear. Furthermore, little is known about the distribution of LEA proteins in multicellular organisms or tissue-specific requirements in conferring stress protection. Here, we used the nematode *C. elegans* as a model to study the endogenous function of an LEA protein in an animal. We created a null mutant of *C. elegans* LEA-1, as well as endogenous fluorescent reporters of the protein. LEA-1 mutant animals formed defective dauer larvae at high temperature. We confirmed that *C. elegans* lacking LEA-1 are sensitive to desiccation. LEA-1 mutants were also sensitive to heat and osmotic stress and were prone to protein aggregation. During desiccation, LEA-1 expression increased and became more widespread throughout the body. LEA-1 was required at high levels in body wall muscle for animals to survive desiccation and osmotic stress, but expression in body wall muscle alone was not sufficient for stress resistance, indicating a likely requirement in multiple tissues. We identified minimal motifs within *C. elegans* LEA-1 that were sufficient to increase desiccation survival of *E. coli*. To test whether such motifs are central to LEA-1's in vivo functions, we then replaced the sequence of *lea-1* with these minimal motifs and found that *C. elegans* dauer larvae formed normally and survived osmotic stress and mild desiccation at the same levels as worms with the full-length protein. Our results provide insights into the endogenous functions and expression dynamics of an LEA protein in a multicellular animal. The results show that LEA-1 buffers animals from a broad range of stresses. Our identification of LEA motifs that can function in both bacteria and in a multicellular organism in vivo suggests the possibility of engineering LEA-1-derived peptides for optimized desiccation protection.

1080A **Different gametogenesis states uniquely impact longevity in *C. elegans*** Amaresh Chaturbedi[1], Bennett W. Fox[2], Frank C. Schroeder[2], Siu Sylvia Lee[11]Department of Molecular Biology and Genetics, Cornell University, [2]Boyce Thompson Institute, Cornell University

Curtailed reproduction affects lifespan and fat metabolism in diverse organisms, suggesting the existence of a regulatory axis between these processes. However, much remains to be learned about the molecular basis of this connection. In *Caenorhabditis elegans*, previous studies have highlighted that the complete removal of germ cells leads to an extended lifespan and increased fat accumulation (Hsin and Kenyon, 1999). The hermaphroditic germline of *C. elegans* provides an opportunity to investigate the impact of different types of germline anomalies on longevity and fat metabolism. In this study, we compared the lipidomic, transcriptomic, and phenotypic differences in three sterile mutants, including the germline-less *glp-1*, feminized *fem-*

3, and masculinized *mog-3*. Our results showed that although all three self-sterile mutants accumulate excess fat, *glp-1* mutants are consistently long-lived, *fem-3* mutants are long-lived only at specific temperature, and in contrast, the *mog-3* mutants are consistently short-lived. We found that these self-sterile mutants share similar lipid profiles and overlapping transcriptomic changes, but they also show specific differences. Our integrated analysis of the lipidomic and transcriptomic data revealed a coordinated alteration of the sphingolipid metabolism pathway in the self-sterile mutants. Our genetic analysis revealed that the transcription factor DAF-16/FOXO plays a key role in the full longevity of the *glp-1* and *fem-3* mutants but is dispensable for the shorter lifespan of the *mog-3* mutant. Moreover, we found that disrupting sperm maturation partially restores the shortened lifespan of several masculinized mutants, indicating that excess sperms in an otherwise hermaphroditic soma may limit their lifespan. In conclusion, our study suggests that disruptions of various germ cell populations lead to distinctive and intricate physiological and longevity outcomes. These findings highlight promising avenues for further research.

1081A **Identifying core gene regulatory networks involved in host-gut bacterium interactions in *C. elegans*** Alejandra Zarate Potes[1], Irtiqa Ali[2], Laila Rees[2], Hayley Brownless[2], Margarida Camacho[2], Eleanor Crago[1], Jack Martin[1], Natália S. Jardim[1], Rajal Patel[1], Leah Catchpole[3], Karim Gharbi[3], Alexandre Benedetto[11]Lancaster University, [2]Biomedical and Life Sciences, Lancaster University, [3]Earlham Institute

Animal health is majorly influenced by microbial commensals with impact on metabolism, behaviour, disease, response to treatments and ageing. To develop interventions that promote life-long health, understanding the complex interplay of molecular pathways that regulate these interactions is critical.

To address this challenge, we first conducted a metanalysis of available RNAseq datasets to identify host genes most frequently differentially regulated upon microbial exposure. We then reasoned that studying naïve worms' early exposure to Gram+ or Gram- gut pathogens would reveal core gene regulatory networks involved in host-gut microbe communication beyond contexts of infection. Using deadly gut pathogens would also enable pangenome host genetic screening via simple automated survival assays (LFASS).

We thus adopted a multiomics approach, performing paired host-microbe transcriptomics, proteomics, and targeted metabolomics of wild type N2 and *daf-2* mutant worms exposed to *E. coli* OP50 control, *Enterococcus faecalis* OG1RF or *Pseudomonas aeruginosa* PA14 for 2, 4 ,6 and 12h. To enable analysis of concomitant host and microbe transcriptional changes we developed a dual RNA extraction method yielding ~5% bacterial and ~95% host sequencing reads. We combined these approaches with parallel whole-genome phenotypic screens on *rrf-3* mutants using the Ahringer RNAi clone library.

Currently, we have completed the genetic screen for chromosomes X, I, II, III, yielding expected hits such as the GATA transcription factor *elt-2*, and hedgehog-like signalling genes such as *grl-21*, *ptr-18* and *qua-1* that were flagged in previous studies. Analysis of host gene expression changes so for identified new genes of interest, including the HN4g orthologue and predicted immunoregulator *nhr-112*, with knockdown of *nhr-112* impacting the kynurenine pathway and resulting in hypersensitivity to PA14 but increased resistance to OG1RF.

Here we will be presenting our methodologies and progress to date, including novel insights from our ongoing genetic screens, transcriptomics, proteomics, and targeted metabolomics analyses relevant to innate immunity and ageing.

1082A **Dietary bacteria reduce *C. elegans* fat content via pathways converging at phosphotidylcholine** Hsiao-Fen Han[1], Shao-Fu Nien[1], Chia-Yi Chiang[1], Man-Tzu Li[1], Lien-Chieh Lin[1], Yu-Shiuan Lin[1], Chao-Wen Wang[2], Yi-Chun Wu[1,31]Institute of Molecular and Cellular Biology, National Taiwan University, [2]Institute of Plant and Microbial Biology, Academia Sinica, [3]Institute of Atomic and Molecular Sciences, Academia Sinica

Diets provide nutrients and energy for organisms. Imbalanced diets have been linked to metabolic disorders, including obesity, diabetes, and cardiovascular diseases. Nevertheless, the mechanisms by which organisms respond to different dietary compositions to modulate biological processes and physiological conditions remain poorly understood. Here, we fed *C. elegans* with two dietary bacterial strains *C. aquatica* DA1877 (DA) and the standard *E. coli* OP50 (OP) to investigate the diet and host interactions. We found that the DA diet alters lipid homeostasis in *C. elegans*, resulting in decreased lipid content, as well as smaller and fewer lipid droplets in the intestine. To understand the molecular mechanisms underlying these changes, we integrated lipidomic and transcriptomic analyses, and identified the repression of delta-(9) fatty acid desaturase *fat-7* as a key factor in the DA diet-induced lipid metabolic reprogramming. A genetic screen using *fat-7::gfp* as a diet-mediated lipid metabolism sensor and epistasis analyses revealed the involvement of genes in B12 metabolism and the *sams-1*-phosphatidylcholine (PC) axis in regulating lipid metabolism in response to diets. We found that the DA diet enhances PC levels and that the transcription factor SBP-1 acts downstream of the B12-*sams-1*-PC axis to repress *fat-7* expression and lipid content. Interestingly, our transcriptomic analysis also revealed the significant up-regulation of acid sphingomyelinase *asm-3*, which produces the intermediate metabolite phosphocholine in the PC synthesis pathway in DA-fed worms. Further genetic experiments demonstrated that *asm-3* constructs

a positive feedback loop that potentiates PC level, and that ASM-3 is secreted from the intestine and enters coelomocytes to regulate diet-mediated lipid content change. Altogether, our data demonstrated that *C. elegans* utilizes the *sams-1*-PC axis to regulate lipogenesis in response to dietary metabolite B12. Our findings uncovered the role of coelomocytes in regulating diet-dependent lipid metabolism via the signaling of *asm-3* from the intestine.

1083A **Age-dependent associations between gut microbiota composition host gene expression.** Rahul Bodkhe[1], Rebecca Choi[2], Kenneth Trang[3], Michael Shapira[31]Integrative Biology, UC Berkeley, [2]integrative biology, University of California. Berkeley, [3]Integrative Biology, University of California, Berkeley

Keywords: Microbiome, aging, gene expression, Enterobacteriaceae

Aging is a complex process that involves the gradual deterioration of physiological, metabolic, and immunological functions. Recent research in our lab has identified age-dependent changes in gut microbiota composition, which have been associated with decreased resistance to infection. This suggested that changes in microbiome composition accompanying host aging could in turn affect some aspects of host aging. To begin addressing cause and effect relationships between host aging and age-dependent changes in microbiome composition, we characterized gut microbiome composition concomitant with host gene expression in aging worms raised on a defined community of 20 worm gut commensals. NextGen sequencing of the V4 variable region of the 16S rRNA gene was used for microbiome characterization and RNA-Seq was used to characterize host gene expression. Microbiome characterization by sequencing and fluorescence imaging of two fluorescently *tagged* community members (*Enterobacter hormachei* CEent1-dsRed and *Enterobacter ludvigii* CEent3-GFP) revealed an age-dependent increase in the abundance of *Enterobacteriaceae*, and a concomitant decrease in that of *Bacillaceae*. RNA-seq analysis in turn identified 163 transcripts that were significantly upregulated in aging worms raised on SC20 (with a p-value < 0.05 and a log fold-change > 1.5) compared to worms raised on *E. coli OP50* and 303 that were downregulated. Among the upregulated genes we found enrichment for immune genes, represented by annotations such as defense response to bacterium or defense response to Gram-negative bacterium; among the downregulated gene we found enrichment for "cellular response to xenobiotic stimulus and xenobiotic metabolic process. These differential gene expression patterns and more would be discussed.

1084A **Effects on healthspan of exposure to environmental pollutants causing mitochondrial DNA damage and depletion** Javier Huayta, Joel N. Meye rNicholas School of the Environment, Duke University

Mitochondrial dysfunction is considered a hallmark of aging, and is a characteristic of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and ALS, as well as cardiovascular diseases, diabetes, fatiguing illnesses, sarcopenia, and cancer. A large number of environmental toxicants such as polycyclic aromatic hydrocarbons, air pollutants, heavy metals, and pesticides are increasingly present in waterways, air, food, homes, and workplaces, leading to a higher risk of exposure and development of chronic disease. Many of these chemicals cause mitochondrial DNA (mtDNA) damage, or cause mtDNA depletion by reducing mitochondrial copy number (mtCN), which is an intriguing observation given the strong association of mitochondrial dysfunction with healthspan.

Aging research has not resolved whether mtDNA damage and depletion contribute to the aging process, or are outcomes of aging. Moreover, the impact of mtDNA damage in aging has been examined in the context of lifespan, but not by observing molecular and physiological phenotypes at the tissue or cellular level. Studying these outcomes should prove relevant to determine the role of mtDNA damage and depletion in aging. We hypothesize that exposure to environmental pollutants that cause mtDNA damage and depletion, at concentrations relevant for environmental human exposure, will contribute to a reduction of healthspan over the lifespan.

C. elegans is already a well-established model organism for both aging and toxicology —but has yet to be exploited to test the intersection between chemical exposure and aging. Mitochondria are present in higher numbers in muscles relative to most other tissues. Therefore, we will assess physiological changes in the pharynx, and changes in locomotion. Our results indicate a decrease in pumping rate and deterioration of pharynx morphology in C. elegans exposed to ultraviolet C (UVC) light, and ethidium bromide (EtBr). UVC produces mtDNA damage and EtBr causes mtCN depletion. To improve our understanding of these effects, we will use chemical concentrations relevant to human environmental exposure. Additionally, we will perform interventions aimed to offset the effects of mtDNA damage and mtCN depletion. If successful, this will provide mechanistic information and illustrate potential translational therapeutic actions.

1085A **Using C. elegans to study the interaction of microbiome and parasitic worms' anaerobic metabolism** Marina Musa, Andrew Fraser Molecular Genetics, University of Toronto, Donnelly Centre for Cellular and Biomolecular Research

Parasitic worms such as hookworm and whipworm infect over a billion people worldwide — they are major pathogens. With only a small number of anthelminthics available and rising resistance in multiple species, we need new strategies to treat these

infections. The complex lifecycle of parasitic worms creates a unique target for treatment; once these worms infect the host's gut, they must adapt to its low oxygen environment. To do this, parasites switch to an unusual form of anaerobic metabolism that requires the electron carrier rhodoquinone (RQ). RQ-dependent metabolism is critical for parasite survival inside the host. Crucially, RQ is not produced or used by humans. Blocking RQ synthesis or utilization would therefore kill the parasite while not affecting the host. We found that bacterial diet affects the worms RQ-dependent metabolism; to elucidate exactly how bacteria affect the RQ metabolism, we screened the Keio collection, a library of nearly 4000 single deletion *E. coli* mutants. Our results suggest bacterial iron metabolism plays a crucial role in the RQ metabolism of the worms that appears to be more complex than simple iron availability or deficiency. In particular, we found two bacterial genes, fiu and ybiX, which are involved in iron metabolism and whose deletions have opposite effects on the worms RQ metabolism. Studying these and similar genes shows potential not only to tell us more about nutritional requirements of the parasites in the gut, but mechanisms of uptake of iron, a major limiting micronutrient, in general. Most importantly, it suggests that altering the gut microenvironment may allow us to modify the metabolism of parasitic worms in a way that would decrease the parasitic load. We hope that this will open up a new area for treatment of these major human pathogens.

1086A **Heat shock stress elicits a defined metabolomic response** Alexander Munden[1], Amy K Walker[1], Dana Miller[21]Program in Molecular Medicine, University of Massachusetts Chan Medical School, [2]Biochemistry, University of Washington

The heat shock response is a conserved mechanism by which cells respond to a variety of stresses through upregulating genes involved in chaperoning and proteostasis to ameliorate the cellular environment and resolve protein misfolding. The role of metabolism and how the metabolome responds to heat stress remains obscure. Our lab has recently linked depletion of specific S-adenosylmethionine (SAM) synthases to dramatic changes in the capacity of *C. elegans* to survive heat shock. Loss of *sams-1* led to increased survival under heat shock, while loss of *sams-4* surprisingly led to enhanced survival. While we identified transcriptome and epigenetic changes that may account for the disparate responses, SAM synthases also play an important role in one-carbon metabolism (1CC). Therefore, to address whether the metabolome changes in response to heat shock response, we performed targeted metabolomics on *C. elegans* with and without heat shock combined with RNAi on *sams-1* and *sams-4*. We have identified several metabolites altered in response to heat shock, as well as those that change specifically upon knockdown of *sams-1* or *sams-4*. This analysis suggests that heat shock initiates a unique metabolic state and that the two SAM synthases have distinct impacts on this stress response.

1087A **High-throughput drug screening in *C. elegans* uncovers new classes of anthelmintics targeting rhodoquinone-dependent metabolism** Xenia Serrat, Taylor Davie, Andy Fraser Donnelly Centre (University of Toronto)

Helminths infect over one billion people and cause significant economic losses in agriculture worldwide. Only a few classes of anthelmintics are currently available and resistance to all of them has already arisen in veterinary parasites, while it threatens the control of human helminthiases. To address the need for new anthelmintics with alternative mechanisms of action, we focus on the adaptive metabolism used by helminths in low-oxygen environments such as the host intestine. There, helminths rely on rhodoquinone (RQ) to generate energy and since this electron carrier is absent in host species, RQ is a promising target. Our lab developed specific assays to study RQ-dependent metabolism (RQDM) in the free-living helminth *C. elegans,* which allowed the identification of key enzymes for RQ biosynthesis. These assays include an image-based movement assay to screen for RQDM inhibition and a novel fluorescence-based assay to specifically assess inhibition of the kynurenine pathway, which provides the precursors for RQ biosynthesis.

We have used these assays to test 50,000 commercially available compounds for their ability to block either RQDM or RQ synthesis in *C. elegans,* leading to the identification of several different structural classes of active compounds. We are currently studying the mechanism of action of the top active compounds and working toward the identification of their targets. This pipeline will uncover new classes of selective anthelmintics, which will be finally tested in murine models of helminth infection. Besides addressing a major problem in human health, our work provides new biological insights into the unique metabolic strategies of parasitic helminths.

1088A **Distinct Effects of Diverse Bacteria on the Transmission of Orsay Virus in *C. elegans*** Brian G Vassallo[1,2,3], Noemie Scheidel[1,2], Sylvia E. J. Fischer[1,2], Dennis H. Kim[1,21]Division of Infectious Diseases, Boston Children's Hospital, [2]Department of Pediatrics, Harvard Medical School, [3]Department of Biology, MIT

The determinants governing transmissibility of viruses between susceptible hosts are not well understood. The discovery of Orsay virus and the experimental tractability of *C. elegans* host enable the study of the determinants of virus transmission in a simple host. The presence of the microbiome has been shown to enhance viral infection in mice. However, species-specific bacterial effects on virus transmission have not been studied in detail. Here we used *Caenorhabditis elegans* and Orsay virus to screen a panel of individual bacteria from *C. elegans'* natural environment to study their impact on virus transmission rates. We identified that exposure to and ingestion of these bacteria result in divergent bacteria-specific effects on transmission rate. Specifically, we

observed that the presence of *Pseudomonas lurida* reduced transmission and infection rates relative to the standard laboratory diet of *E. coli* OP50, whereas the presence and ingestion of *Ochrobactrum* species enhanced infection by Orsay virus. We further examined the effects of *Pseudomonas aeruginosa* strains that have been previously characterized together with the *C. elegans* host, and we observed that pathogenic *P. aeruginosa* PA01 and PA14 strains markedly attenuated infectivity of Orsay virus. We observed that the inhibitory effect of *P. aeruginosa* strains was dependent on genetic pathways in *P. aeruginosa* regulating virulence and quorum-sensing pathways. Our data suggest there is a tripartite interaction between the Orsay virus, bacteria, and the *C. elegans* host that can strongly modulate virus transmission in a bacterial species-specific manner.

1089A **Regulation of *C. elegans* longevity by sodium** Franziska Pohl, Brian M Egan, Kerry Kornfeld Washington University in St. Louis

C. elegans is a leading model system to study organismal aging. Most aging experiments are conducted in standard culture conditions: Nematode Growth Medium (NGM) agar with a lawn of *E. coli* OP50. There are many advantages of widespread use of standard culture conditions; most importantly, effects of genetic, pharmacologic, and temperature manipulations can be readily compared between labs. However, it has become clear that certain aspects of standard culture conditions influence aging and lifespan, and it is valuable to document these effects. For example, *E. coli* OP50 is mildly pathogenic to *C. elegans*, and culture with this live bacteria moderately shortens lifespan. Here we present evidence that another component of standard culture conditions can also influence lifespan: NaCl included in the NGM medium. The Kornfeld lab previously reported that captopril, an angiotensin-converting enzyme (ACE) inhibitor used to treat high blood pressure in humans, increases life span in *C. elegans*. Because high blood pressure is strongly associated with excess dietary sodium, we investigated how NaCl affects *C. elegans* lifespan and interacts with captopril. In *C. elegans*, osmolarity is sensed by amphid neurons in the head, including ASH and ASE, and worms avoid high osmolarity - a behavior called osmotic avoidance. We discovered that removing supplemental NaCl from the NGM significantly extended worm lifespan; brood size was not significantly affected, suggesting this is not a dietary restriction phenomenon. This life span increase was also observed in long-lived *daf-2(lf)* mutants, suggesting NaCl removal may function by a different pathway from the insulin/insulin-like growth factor (IGF) signaling pathway. When confronted with high osmolarity, worms synthesize and accumulate high levels of the organic osmolyte glycerol, a small polyol that is protective. Transcriptional induction of glycerol-3-phosphate dehydrogenase-1 (*gpdh-1*) is at least partially responsible for glycerol synthesis during hypertonic stress. We discovered that *gpdh-1* displays age-related accumulation, raising the possibility that there is an age-related susceptibility to NaCl stress. We conclude that supplemental NaCl added to NGM is a component of standard culture conditions that influences lifespan. This observation may lead to new insights into the control of age-related degeneration by the osmotic environment and homeostatic systems that respond to osmotic fluctuations.

1090A **Decreased spliceosome fidelity inhibits mTOR signalling and promotes longevity** Wenming Huang[1,2], Adam Antebi[11]Max Planck Institute for Biology of Ageing, [2]1Max Planck Institute for Biology of Ageing

Ageing is associated with dysregulated mRNA splicing. However, the functions of spliceosome and splicing factors in ageing remains largely unclear. By using *Caenorhabditis elegans* as model, we have uncovered a novel longevity pathway mediated by RNP-6/RBM-39 spliceosomal complex and mTOR signaling. Genetic and biochemical evidence show that RNP-6/RBM-39-mTORC1 signaling axis is partially mediated by *egl-8* intron retention in neuronal tissues. Interestingly, PUF60 downregulation in mammalian cells also potently and specifically inhibits mTORC1 signaling. Taken together, our findings provide novel insights into the mechanisms of splicing factor in ageing and shed light on delaying ageing through targeting spliceosome.

1091A **BAZ-2 is a negative regulator of cell non-autonomous proteostasis via acetylcholine signalling** Christian Gallrein, Ashley Williams, Bjoern Schumacher Institute for Genome Stability in Aging and Disease, CECAD Research Institute, University and University Hospital of Cologne

Accumulation of aggregation prone proteins and peptides, such as Amyloid-beta or poly-glutamine, can lead to age-dependent neurodegenerative disorders. Here, we investigated how systemic neuronal signalling mechanisms could impact protein aggregation and neurotoxicity. We focused on the *baz-2* gene, which previously has been implicated in regulating healthy ageing in *C. elegans* and mice. *baz-2* mutants have been shown to have an improved mitochondrial unfolded protein response (UPRmt), rendering nematodes more resistant to diverse stressors, and preventing age-dependent cognitive decline in mice. In humans, ageing and Alzheimer's Disease are positively correlated with *BAZ2B* (a homolog of *baz-2*) expression.

Here, we investigated whether *baz-2* depletion could ameliorate protein aggregation in nematode models of neurodegenerative diseases. We monitored Amyloid-beta (as model of Alzheimer's Disease) or poly-glutamine (as model of poly Q diseases) aggregation upon depletion of *baz-2*. We used fluorescence lifetime microscopy to score aggregation and analysed the correlation with lifespan and motility as read-out of vitality. Depletion of *baz-2* led to a reduction of protein aggregation, as well as, an alleviation of amyloid-induced toxicity. We observed and characterised a cell non-autonomous mode of BAZ-2 signalling that is

dependent on acetylcholine and the endoplasmic reticulum unfolded protein response.

1092A **Identification of neuronal and epidermal determinants of immune signaling underlying oomycete recognition in *C. elegans*** Manish Grover, Florence Drury, Kenneth Liu, Michalis BarkoulasLife Sciences, Imperial College London

Innate immune responses can be triggered by pathogen-associated molecular patterns or damage-related host biomolecules, which are detected by specific host receptors present in specialized immune cells, such as macrophages and dendritic cells. In the absence of such cells in *C. elegans*, neuronal and epithelial cells play a role in recognition and defense in a pathogen-specific manner, however the exact machinery underlying these processes often remains elusive. We recently identified oomycetes as natural pathogens of *C. elegans* and described a protective transcriptional program that is likely activated by neuronal recognition of the pathogen leading to induction of multiple *chitinase-like (chil)* genes in the epidermis. CHIL proteins enable animals to modify their cuticle and thereby antagonize infection by reducing oomycete attachment. We performed a forward genetic screen to identify the molecular determinants of this neuron-to-epidermis signaling underlying oomycete recognition. We report that the response is initiated by activation of the C-type lectin receptors CLEC-27/CLEC-35 in chemosensory neurons, which are encoded by adjacent genes in the genome sharing the same promoter. Neuronal recognition is followed by activation of a kinase-pseudokinase pair in the epidermal membrane formed by OLD-1 and FLOR-1 respectively leading to the induction of *chil* genes in the epidermis. We also present that the PAX6 homolog VAB-3, commonly studied for its conserved role in animal visual system development, regulates *old-1* gene expression, and consequently the response to oomycete recognition. Overall, our findings shed light on how neuron-to-epidermis communication shapes the nematode defense against natural oomycete infection.

1093A **Mitochondrial import of non-coding RNAs regulates stress resistance and longevity in *C. elegans*** Emiko Okabe, Masaharu Uno, Eisuke NishidaLaboratory for Molecular Biology of Aging, RIKEN Center for Biosystems Dynamics Research

Mitochondria are organelles essential for producing the cellular energy to maintain cellular and systemic homeostasis. Mitochondrial dysfunction is implicated in aging and aging-related diseases. Although mitochondria possess their own genome, most mitochondrial proteins are encoded in the nuclear genome. Nuclei can modulate mitochondrial activity and promote mitochondrial biogenesis through the regulation of mitochondrial protein gene expression. Conversely, mitochondria are capable of altering the expression of nuclear genes in response to changes in cellular conditions. Therefore, nuclei and mitochondria constantly communicate with each other in order to ensure cellular and systemic homeostasis. Recently, it has been suggested that non-coding RNAs (ncRNAs) act as the signaling molecules that mediate communication between the nuclei and mitochondria. However, it is not clear whether mitochondrial-nuclear communications via ncRNAs are involved in the regulation of lifespan. Here, we have found that two RNA-binding proteins, PNPASE and LRPPRC, regulate stress resistance and longevity. Knockdown of each of these two genes increases resistance to oxidative and osmotic stress, and extends the longevity. This longevity phenotype requires the DAF-16/FOXO transcription factor. Furthermore, knockdown of each of two nuclear-encoded ncRNAs, RNase P RNA and MRP RNA, which are shown to be imported into mitochondria by PNPASE, extends lifespan. These results suggest that mitochondrial-nuclear communications via ncRNA play an important role in the regulation of longevity. We are now examining the underlying mechanisms of these phenomena.

1094A **DAF-16 is required specifically during arrest to allow fast recovery from L1 starvation** Marta Munoz-Barrera, Almudena Moreno-Rivero, Alejandro Mata-Cabana, Maria Olmedo Genetics, University of Seville

Developmental arrest of *C. elegans* at the L1 stage is an emerging model for the study of cellular quiescence and reactivation. During arrest, L1 larvae undergo a process that shares phenotypic hallmarks with ageing. When arrested L1 encounter food, cell divisions resume and postembryonic development proceeds. Remarkably, the markers of aging accumulated during arrest are erased upon feeding of the arrested larvae. Combining a novel method to measure postembryonic development with the direct analysis of cell divisions, we have observed that prolonged L1 quiescence delays reactivation of blast cell divisions in *C. elegans*, leading to a delay in the initiation of postembryonic development. Insulin signalling modulates the rate of L1 ageing, affecting proliferative potential after quiescence. This role of reduce insulin signalling requires the activation of the transcription factor DAF-16. At the time, we speculated how DAF-16 activation could be relevant at different moments of the process to promote recovery from the regulation of maternal provisioning, to arrest or to recovery itself. In the present work, we are studying the temporal and spatial requirements for insulin signaling in the control of L1 quiescence and recovery. Using an approach based on the analysis of the effect of maternal and zygotic genotypes we have restricted the timing of insulin signaling sensitivity to the same generation that experiences the larval arrest. Furthermore, using a combination of interference by RNAi and protein-specific degradation, we have determined that DAF-16 is required specifically during L1 arrest to maintain the potential to proliferate of the quiescent larvae.

1095A **Bacteria-derived RNA and inter-tissue communication promotes proteostasis in *C. elegans*** Emmanouil Kyriakakis, Chiara Medde, Anne Spang University of Basel

Life expectancy has greatly increased in the last decades. As we age, a progressive decline of physiological functions occurs, leading to a multitude of diseases. Besides genetic composition, environmental and nutritional factors influence both health- and lifespan. Diet is thought to be a major factor for healthy ageing. However, the constituents of a balanced diet and how it affects individuals remain highly debated. Over the years, *C. elegans* has been proven to be an ideal model for health and lifespan studies with major discoveries being confirmed across species. To answer how diet and which dietary components influence cellular and organismal fitness and lifespan in a reliable and expeditious way, we studied *C. elegans* and its bacterial diet. We find that a mixed diet of two *E. coli* strains promotes *C. elegans* fitness and that bacterially-expressed ribonuclease 3 influences the accumulation of protein aggregates in *C. elegans* body-wall muscles, *via* a cell-non autonomous mechanism involving intestinal uptake of bacterial-derived RNA species, the RNAi machinery and autophagy. Overall, our findings provide significant advancements on the mechanisms on how dietary cues influences organismal fitness and promote proteostasis in *C. elegans*.

1096A **5-Fluorouracil enhances cold survival by inducing alternative protein turnover pathways** Abhishek Anil Dubey, Wojciech PokrzywaII MCB, Warsaw

Hibernation is an adaptive strategy used by various organisms to survive hostile environmental conditions, such as low temperatures. One conserved feature of hibernation is the global repression of translation. Using Caenorhabditis elegans, which exhibits hibernation-like behavior, we found that proteasome composition and activity disruption significantly reduces low-temperature survival. However, exposure of worms to 5'-fluorodeoxyuridine (FUdR) and its derivatives, or depletion of their primary target thymidylate synthetase (TS), desensitizes animals to cold. We show that FUdR increases proteasome processivity and even permits proteasome function in the absence of some subunits or drug inhibition. Our data indicate that FUdR-induced mechanism(s) do not depend on autophagy, GLP-1, FEM-1, RPN-6.1, or DAF-16 but partially require SKN-1-dependent transcription. Finally, we show that FUdR enhances repression of protein synthesis while allowing specific transcripts to bypass translation inhibition to trigger a cell non-autonomous stress response inducing pathways that promote the survival of cold-sensitive tissues such as neurons. Our findings provide a new foundation for comprehending cellular adaptation to hypothermia, which may have biomedical implications, as well as a better understanding of the TS-dependent mechanism enhancing protein turnover and organismal proteostasis.

1097A ***tts-1* lncRNA represses *daf-2* mutant longevity in *C. elegans*** Emily Mathew[1], Benjamin McCarthy[1], Evan Lister-Shimauchi[2], Shawn Ahmed[11]Genetics, The University of North Carolina at Chapel Hill, [2]The University of North Carolina at Chapel Hill

The 711 nucleotide non-coding RNA *tts-1* is the most highly expressed RNA in *C. elegans* dauer larvae (1). *tts-1* was named *telomere transcribed sequence-1* because it contains several telomere repeats. The *trt-1* telomerase reverse transcriptase maintains telomere length in *C. elegans* (2), but the non-coding telomerase RNA subunit that contains the template for the $(TTAGGC)_n$ nematode telomere repeat sequence is not known. We asked if *tts-1* might be the *C. elegans* telomerase RNA by deleting *tts-1* using CRISPR/Cas9 genome editing. However, *tts-1* mutants did not display transgenerational sterility and telomere fusions observed in *trt-1* telomerase mutants.

tts-1 RNA is very highly expressed in long-lived *daf-2* insulin/IGF-1 signaling mutants, and RNAi knockdown of *tts-1* was reported to shorten *daf-2* mutant lifespan (3). Instead, we found that *daf-2; tts-1* double mutants displayed double the lifespan of *daf-2* single mutants, consistent with a recent report (4). In addition, we found that *tts-1* did not affect dauer formation for *daf-2; tts-1* double mutants. We used RNA FISH to show that *tts-1* is expressed in intestinal and neuronal nuclei and often localizes to prominent foci. Non-coding RNAs that occupy discrete locations within nuclei are known to regulate epigenetic gene expression. Given the strong *tts-1* expression observed in *daf-2* mutant nuclei in comparison to wildtype controls and that several histone modifying methyltransferases repress *daf-2* mutant longevity (3), we suggest *tts-1* RNA may possess a nuclear epigenetic function. We conclude that the most highly expressed RNA in dauer larvae may act in the nuclei of neurons or intestinal cells to markedly repress the longevity of *daf-2* insulin/IGF-1 signaling mutants. We hope to understand ways in which *tts-1* may be relevant to other aspects of lifespan and in the progression of age-related disorders such as Alzheimer's disease.

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1098A **Functions of Chloride intracellular channel proteins EXL-1 in Heat Stress in *C. elegans*** Jun LiangScience, Borough of

Manhattan Community College, CUNY

Chloride intracellular channel proteins (CLIC) are highly conserved multifunctional proteins. Cellular stress molecules induce endogenous mammalian CLIC to translocate from the cytoplasm into the nucleus. It has been reported that Mammalian Schnurri-2 is required for CLIC nuclear translocation in response to Transforming Growth Factor – beta. Schnurri-2 is a transcription cofactor in the BMP pathway. However, how the CLIC genes regulate thermotolerance is largely unknown. To address these issues, we took advantage of viable CLIC mutants in *C. elegans* and characterized its functions in heat stress. Schnurri-2 is homologous to *C. elegans* SMA-9 which functions in the DBL-1/TGF-beta pathway. We analyzed integrated EXL-1::GFP lines in wild type background and observed strong fluorescence in intestinal cells. EXL-1::GFP is translocated into the nucleus under heat shock conditions. Supporting functional importance of this, *exl-1* loss-of-function mutants are thermo-sensitive. Furthermore, we generated double mutants of *exl-1* and the DBL-1/TGF-beta pathway components. To our surprise, the double mutants survived better than any single mutants. To elucidate molecular mechanism of *exl-1* regulated heat stress, we measured EXL-1::GFP intensity under various DBL-1 pathway mutants background. EXL-1::GFP nuclear translocation level under heat stress has significantly reduced in loss of function mutants background. However, these phenomena only stay true for the first a few hours under heat shock. Long-term exposure to heat abolishes these differences. In addition, we introduced RAD-SMAD (a DBL-1 pathway transcriptional marker) into *exl-1* mutants background, however RAD-SMAD activities were not affected by heat stress. Thus, *exl-1* does not regulate the DBL-1 pathway target gene transcription. We also evaluated the lifespan of the *exl-1* mutants; we found no significant difference from wild type animals. Thus, *exl-1* regulation of heat stress response is specific, not related to its lifespan. In order to further understand *exl-1* regulated heat stress management, we are investigating various downstream target genes in heat shock response.

1099A **Identifying bacterial genes and pathways controlling intracellular filamentation during *B. atropi* infection** Serena J Meadows-Graves, Tuan D Tran, Robert J Luallen Biology, San Diego State University

The short life span and transparency of lab nematodes are just a couple of attributes that make them a great research organism, especially with regards to host-microbe interactions. We previously found that a wild-isolated strain of *Oscheius tipulae* nematodes from Finistère, France contained the first discovered intracellular bacterium to infect free-living nematodes. This bacterium, *Bordetella atropi,* spreads through the host intestines using a strategy of morphological change called filamentation, whereby a bacterium divides without septation to form filaments that span across multiple intestinal cells. Using a filamentation-deficient mutant of *B. atropi*, we found that filamentation is required for cell-to-cell spreading and determined that a bacterial metabolic pathway to produce UDP-glucose served as an indication of rich conditions to initiate filamentation.

In order to discover other bacterial genes and pathways required for in vivo filamentation, we plan to conduct a large-scale, non-redundant screen using a defined transposon mutagenesis library. As a proof-of-concept, we have been able to isolate a *B. atropi* filamentation mutant using a highly enriched media (Terrific broth) and the antibiotic Cefotaxime to induce in vitro filaments and using 5 µm PVDF filtration for a screen. For the in vivo screen, we intend to disrupt 50-60% of the predicted 5,522 protein coding genes in the *B. atropi* genome. We have created a transgenic strain of *B. atropi* integrated with a red fluorescent protein (tdTomato) via the Tn7 transposon to be used to create the mutant library. From this defined mutant library we will create a nonredundant library of about 2700-3400 unique mutants. This nonredundant library will then be used to screen for in vivo filamentation mutants. We infect the *O. tipulae* strain JU1501, the wild-isolate from which *B. atropi* was discovered, with the mutagenesis library and screen for filamentation mutants by measuring Relative Fluorescence Units (RFUs). We hypothesize that wild type *B. atropi* will increase in fluorescence at a slow rate until a later time point when fluorescence should increase dramatically, as spreading filaments are growing and diluting the fluorescence signal until they septate at late infection and fill the entire intestine. By contrast, filamentation mutants would remain in one cell and replicate quicker, giving strong fluorescence readings at earlier time points. We will use this framework to measure the fluorescence signals of different mutants over a certain time frame to determine if they are capable of filamentation. We expect this screen to hit genes involved in detecting metabolites found in rich conditions, regulating cell division machinery, and the SOS response.

1100A **Complementary CRISPR-based approaches reveal allele and tissue-specific mechanisms of mitochondrial disease** Peter Kropp[1], Andy Golden[21]Biology, Kenyon College, [2]National Institute of Diabetes and Digestive and Kidney Diseases

Rare mitochondrial diseases collectively affect 1 in 5,000 individuals worldwide, however, individual diseases may affect only a few individuals making their study both a challenge and rife with opportunity. Further, mechanistic similarities in disease etiology mean that insights in one disease are typically transferrable to many others. Multiple Mitochondrial Dysfunctions Syndrome 1 (MMDS1) is in the iron-sulphur cluster disease family, but understanding the pathology is complicated by the frequent compound heterozygosity of affected individuals and diverse symptoms. We hypothesized that individual variant alleles likely have phenotypic differences and may even have tissue-specific effects. We modelled patient-specific variations documented in NFU1, the causative factor of MMDS1, in its *C. elegans* ortholog, NFU-1, and studied them as homozygotes to best understand

their individual contributions. The five modelled variations presented as an allelic series resulting in phenotypic heterogeneity, as expected. In focusing on the neuromuscular system, we found that two of these variations have opposing effects altering motility on both solid medium and in liquid. We tested the necessity for *nfu-1* in both neurons and muscles by generating tissue-specific *nfu-1* knockouts which indicated an increased necessity in muscles. In a complementary approach, we then generated tissue-specific re-expression constructs restoring *nfu-1* in either the neurons or muscles of the patient-specific variants. We likewise found that muscle-specific rescue was generally more effective at restoring normal motility than neuronal rescue in both variants. Biochemical analyses indicated that these variants alter acetylcholine signalling, likely increasing sensitivity in the muscles by yet-to-be-determined mechanisms, but knockdown of acetylcholine release from neurons could rescue one, but not both, variants. These findings help clarify the possible outcomes of iron-sulphur cluster dysregulation on neuromuscular function and that genotype-specific therapies could be beneficial for MMDS1 patients amongst other individuals affected by iron-sulphur cluster diseases.

1101A **A Genome-Wide Survey on the Host Factors that Modulate Intestine Permeability** Zachary Markovich, Adriana Abreu, Leah Davis, Yi Sheng, Rui XiaoPhysiology and Aging, University of Florida

The intestine is a main organ of *C. elegans* and is responsible for many evolutionarily conserved functions including food processing, nutrient storage, immunity, and aging. The *C. elegans* intestinal cells form a hollow tube structure and are held together by two apical junctions, functional homologs of the vertebrate intestinal tight junctions. The worm intestine is known to play a central role in aging and pathogen defense. Likewise, the gastrointestinal system (GIS) in humans is key to immune support and homeostasis. Although many human diseases are linked to the leaky gut such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), allergies, autoimmune diseases, diabetes, and even neurodegenerative diseases, there is a large gap in knowledge regarding how the intestinal barrier is established and maintained.

In our pilot study, we have successfully established a streamlined workflow that enables us to efficiently perform genome-wide screening on host factors that modulate intestinal permeability in *C. elegans.* Next, using human colon epithelial cell line and intestinal organoids as *in vitro* human intestinal models, we are examining the evolutionarily conversed roles of *C. elegans* candidate genes in regulating mammalian intestinal barrier permeability. Since many evolutionarily conserved genes and signaling pathways are involved in the intestinal development and function, the knowledge gained from our studies will provide important insights into similar processes in other species.

1102A **Translating the effects of high glucose diets using systems biology approach in *C. elegans*** Chao-Wen Wang, Phebe Chiu Institute of Plant and Microbial Biology, Academia Sinica

Diet is one of the most variable aspects in life. How animals interpret dietary status and adapt their metabolism and physiology accordingly defines an important but extremely complex question. The nematode *Caenorhabditis elegans* is emerging as a powerful model organism for the elucidation of the cellular and molecular mechanism of processes relevant to human health. *C. elegans* fed the standard *Escherichia coli* OP50 diet supplemented with high glucose alters a number of organismal traits, such as body size, developmental rate, and fecundity. Interestingly, we did not observe the same phenotypes when *C. elegans* fed another bacteria diet *Comamonas aquatica* DA1877 supplemented with the same level of high glucose. Here, we take advantage of omics tools to learn about changes underlying the dietary effects in normal and high glucose conditions. Microarray combined with quantitative PCR analyses identified that fatty acid desaturase genes exhibited differential expression patterns in worms fed different high-glucose diets. Lipidomic profiling data revealed a profound change of cis-vaccenic and cyclopropane fatty acids and a trend of reducing phosphatidylcholine and elevating triacylglycerol contents in worms fed the high glucose *E. coli* OP50 diet. By contrast, worms fed the high glucose *C. aquatica* DA1877 diet was able to maintain normal lipid homeostasis as in worms fed *C. aquatica* DA1877 or *E. coli* OP50 diet alone. Although most polar metabolites in worms fed the two diets remained largely unchanged, reducing choline and elevating methionine metabolites was observed only in worms fed high glucose *E. coli* OP50 but not *C. aquatica* DA1877 diets. Together, our findings suggest that the methionine-choline axis is likely the major metabolic route that decipher differential lipid and fat contents, correlated with differential fecundity, when worms fed different high glucose diets. Finally, we provide evidence to show that the diet-specific high glucose effect leading to fecundity changes in worms could be attributed to excess glucose perturbing lipid homeostasis in dietary bacteria.

1103A **Persistent TFIIH binding to DNA damage impairs neuron functionality** Alba Muniesa-Vargas, Carlota Davó-Martínez, Cristina Ribeiro-Silva, Melanie van der Woude, Karen Thijssen, Arjan Theil, Wim Vermeulen, Hannes Lans Erasmus MC

The versatile DNA repair pathway nucleotide excision repair (NER) protects organisms against the harmful effects of helix-distorting DNA damage induced by UV light and diverse metabolism-derived and environmental chemicals. In humans, hereditary mutations that affect the core NER machinery, such as in transcription/DNA repair factor TFIIH, the TFIIH-auxiliary factor XPA or in the endonucleases XPF and XPG, give rise to different diseases. These include the cancer prone disorder xeroderma pigmentosum, the progeroid Cockayne syndrome and the developmental disorder trichothiodystrophy. It

is not understood why mutations in genes that act in the same DNA repair pathway cause these different types of disease.

To understand the pathogenic mechanisms that underlie different NER disorders, we make use of *C. elegans* to investigate mechanisms of DNA repair and their in vivo impact. We show that in both human cells and in *C. elegans*, in the absence of DNA damage excision by XPF and XPG, TFIIH persistently binds to DNA damage. XPA stabilizes TFIIH binding to DNA damage and, consequently, its depletion suppresses the persistent TFIIH stalling in the absence of DNA damage excision. DNA damage causes strong developmental arrest and neuronal dysfunction in *C. elegans* that lack functional XPF or XPG, which is dependent on transcription-coupled NER and XPA-stabilized persistent TFIIH binding to DNA damage. Together, these results indicate that stalled NER intermediates adversely affect cell functionality and organismal development, which provides a plausible explanation for the fact that certain mutations in XPF or XPG cause different human disease features than mutations in XPA.

1104A **Can we untie the linkage between life extension and a decline in healthspan via healthy mitophagy?** Vijigisha Srivastava, Veronica Zelmanovich, Virendra Shukla, Rachely Abergel, Irit Cohen, Shmuel (muli) A Ben-Sasson, Einav Gross Hebrew University of Jerusalem

The most devastating aspect of aging is the debilitating morbidity associated with it. This results from a decline in the proper operation of a critical quality-control system called autophagy, called mitophagy in the case of mitochondrial recycling. The mitophagy process ensures the removal of damaged intracellular organelles and their replacement by newly formed ones. However, mitophagy efficiency progressively declines with age, accumulating damaged mitochondria that contribute to increased oxidative stress and various disease states such as Alzheimer's disease, Parkinson's disease, heart failure, and skeletal muscle weakness. Therefore, increasing mitophagy by pharmacological means is an emerging strategy for treating age-associated conditions.

Studies from recent years show that the natural polyamine spermidine increases autophagy/mitophagy and, in this way, extends life- and healthspan. However, some of its degradation products are harmful, so the use of spermidine as a drug may be limited. To address this, we have developed and tested synthetic polyamine derivatives. These compounds induce mitophagy in the nematode *C. elegans* and mammalian cells and thus protect against oxidative injury and toxic disease proteins. In addition, these Mitophagy Activating Compounds (MACs) lengthen life expectancy and health in *C. elegans*, demonstrating that longevity and healthspan are not mutually exclusive. Finally, our studies provide new insights into the mechanism of action of the MACs and healthy mitophagy. We will leverage this knowledge toward a more rational design of effective MACs to treat age-associated diseases.

1105A **High-Glucose Diet Reduces Male Fertility by Reducing Sperm Size, Competitiveness, and Quality** Kerry A Larkin[1], Jason L Erichsen[1], Marjorie R Liggett[1], Hannah Costa[1], David Chen[1], Caroline D Davis[1], Michael Mastroianni[1], Michelle A Mondoux[21]Biology, College Holy Cross, [2]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* hermaphrodites. We found that high-glucose diet also reduces male fertility in a dose-dependent manner. Concentrations of glucose that have no effect on hermaphrodite self-fertility disrupted mated fertility, which allows us to separate the effects of glucose on males from the effects on hermaphrodites. We tested several aspects of male fertilization success and find multiple defects on a high-glucose diet. First, a high-glucose diet reduces male sperm competitiveness. On a control diet, male sperm is used almost exclusively when males are mated to hermaphrodites. However, on a high-glucose diet, we find that although male sperm are still used preferentially, there was a dose-dependent reduction in the percentage of offspring derived from male sperm, suggesting a reduction in sperm quality. We then measured spermatid size, which is known to correlate with sperm competitiveness, and also found a dose-dependent decrease in male sperm size. We also find a decrease in male spermatid production and embryo viability on a high-glucose diet. However, a high-glucose diet had no effect on spermatid morphology or activation, and small or no effects on mating, mating behavior, and 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.

1106A **Octopamine-MAPK-SKN-1 signaling suppresses mating-induced oxidative stress in Caenorhabditis elegans gonads to protect fertility** Yu Tsai[1], Ying-Hue Lee[2], Yu-Chun Lin[21]Institute of Molecular Biology, Academia Sinica, [2]Academia Sinica

Sexual conflict over mating is costly to female physiology. *Caenorhabditis elegans* hermaphrodites generally produce self-progeny, but they can produce cross-progeny upon successfully mating with a male. We have uncovered that *C. elegans* hermaphrodites experience sexual conflict over mating, resulting in severe costs in terms of their fertility and longevity. We show that reactive oxygen species (ROS) accumulate on the apical surfaces of spermathecal bag cells after successful mating and induce cell damage, leading to ovulation defects and fertility suppression. To counteract these negative impacts, *C. elegans* hermaphrodites deploy the octopamine (OA) regulatory pathway to enhance glutathione (GSH) biosynthesis and protect spermathecae from mating-induced ROS. We show that the SER-3 receptor and mitogen-activated protein kinase (MAPK) KGB-1 cascade transduce the OA signal to transcription factor SKN-1/Nrf2 in the spermatheca to upregulate GSH biosynthesis.

1107A ***C. elegans* TFIIH subunit GTF-2H5/TTDA is a non-essential transcription factor indispensable for DNA repair** Karen Thijssen[1], Hannes Lans[1], Melanie van der Woude[2], Carlota Davó-Martinez[2], Dick HW Dekkers[2], Mariangela Sabatella[2], Jeroen AA Demmers[2], Wim Vermeulen[21]molecular genetics, Erasmus MC Rotterdam, [2]Erasmus MC Rotterdam

The 10-subunit transcription factor TFIIH is vital to both transcription initiation and nucleotide excision repair. In transcription initiation, TFIIH facilitates promoter escape and RNA synthesis by RNA polymerase II. In nucleotide excision repair, TFIIH facilitates DNA damage verification and subsequent excision by endonucleases ERCC1/XPF and XPG. Hereditary mutations in TFIIH subunits cause different diseases, including the cancer prone xeroderma pigmentosum and the progeroid Cockayne syndrome. Mutations in the smallest subunit of TFIIH, TTDA/GTF2H5, cause xeroderma pigmentosum combined with the rare developmental disorder trichothiodystrophy. Trichothiodystrophy is thought to be brought about by gene expression defects, but to which extent TTDA/GTF2H5 is necessary for transcription *in vivo* is unclear. Trichothiodystrophy patients express a partially functional TTDA/GTF2H5 protein whereas mice with complete TTDA/GTF2H5 loss are not viable. Therefore, TTDA/GTF2H5 has been considered to be essential to multicellular life.

We investigated the function of *C. elegans* TFIIH and its GTF-2H5 subunit in transcription and DNA repair. We show that in contrast to full depletion of other TFIIH subunits, complete loss of GTF-2H5 is compatible with *C. elegans* viability and growth. However, GTF-2H5 is indispensable for nucleotide excision repair, in which it promotes recruitment of the TFIIH complex to DNA damage. Also, GTF-2H5 promotes the stability of TFIIH in multiple tissues. Because of this, GTF-2H5 loss causes embryonic lethality when transcription is challenged. These results support the idea that TTDA/GTF2H5 mutations cause transcription impairment that underlies trichothiodystrophy and establish *C. elegans* as potential model for studying the pathogenesis of this disease.

1108A **Regulation of an effector triggered immune response in *C. elegans* against the mitis group streptococci** Anastasiia Ahmedaly, Ashley Persons, Lorraine Byrd, Ransome van der HoevenDiagnostic and Biomedical Sciences, The University of Texas Health Science Center at Houston, School of Dentistry

Recent studies in plants and animals suggest that some effectors can illicit an immune response mainly by disrupting core host processes. This response known as Effector Triggered Immunity (ETI) becomes more relevant in cells lacking a complete range of pattern recognition receptors such as intestinal epithelial cells. In the worm, the BZIP transcription factors ZIP-2 and CEBP-2 mediate an ETI in the intestinal cells in response to Endotoxin A produced by *Pseudomonas aeruginosa*. Recently, we have shown ZIP-2 is activated in the worm in response to hydrogen peroxide (H_2O_2) produced by the mitis group streptococci. The mitis group streptococci are residents of the oral cavity and have shown to be opportunistic pathogens. These microorganisms produce H_2O_2 as a major virulence factor that contributes to their pathogenicity. Using *Streptococcus gordonii* as a representative of the group, we showed that *zip-2* is required for the survival of the worms and activates the expression of the *zip-2*-dependent gene *irg-1*. However, we did not observe a significant change in survival of *cebp-2* mutant worms relative to the wild type worms on *S. gordonii*. This suggests CEBP-2 doesn't contribute to the worm's immune response against *S. gordonii.* Therefore, we investigated if ZIP-2 interacts with other proteins to elicit an ETI response against the mitis group. Using the WormBase interaction partners database, we identified potential candidates that interacted with ZIP-2. We knockdown these candidate genes in N2 worms and worms expressing *irg-1* fused to Green Fluorescent Protein (GFP) and observed the survival and the expression of GFP on *S. gordonii* respectively. Significant increase in survival of *attf-4*, *nhr-68* and *mdt-11* knockdown worms was observed relative to the vector control treated worms. Furthermore, a significant increase in the expression of *irg-1*::GFP was observed in *attf-4*, *nhr-68* and *mdt-11* knockdown worms relative to the vector control treated worms. We also observed *nhr-111* knockdown worms were more susceptible to the pathogen and the expression of *irg-1*::GFP was significantly lower compared to the vector control treated worms respectively. Future studies will focus on characterizing the roles of these proteins in regulating the ETI response to the mitis group streptococci.

1109A **Screening compounds for impacts on innate immunity and inflammatory signaling in Parkinson's Disease in *C. elegans*** Lisa C Benecchi[1], Jake Romley-Murias[2], Tiani B Bello[1], Iris B Farnum[1], Kayla B Rendon-Torres[1], Lili B Kosa[1], Wayne Guida[3], Ray Ball[1], Denise Flaherty[11]Biology, Eckerd College, [2]Chemistry, Eckerd College, [3]Chemistry, University of South Florida

While the adaptive immune system plays an essential role in infection response, the reality is that most organisms use innate immunity to defend themselves against infection and, overall, support inflammation pathways that keep cells healthy. Inflammatory responses can be triggered by pathogens or cell damage and are responsible for initiating the healing process by restoring tissue homeostasis. Parkinson's disease (PD) is an example of a neurodegenerative disease for which inflammation is a hallmark of its pathology. It is characterized by the loss of dopaminergic neurons primarily caused by the aggregation of Lewy Bodies composed of a misfolded and aggregated alpha-synuclein (α-syn). The experiments presented here test the impact of chemical compounds to see if they bolster innate immune pathways in the model organism Caenorhabditis elegans. This was done by examining their effect on the transgenic expression of DAF-16::GFP and PMK-1::GFP, as well as their ability to reduce or exacerbate alpha-synuclein Lewy body aggregation and motility losses in a Parkinsonian model. Our data demonstrate that our compound "PZP" can upregulate DAF-16::GFP expression. Consistent with this finding, PZP also increases α-syn::YFP expression in our Parkinson's model. This suggests that upregulation of DAF-16 can exacerbate alpha-synuclein-based pathology. In a longitudinal study, treatment with our compound "cxl", showed a statistically significant preservation of motility in aged (12 days post egg lay) α-syn::YFP transgenic animals at a 10uM concentration. Furthermore, "cxl" also lowers α-syn::YFP expression in fluorescence intensity in Lewy Body aggregates at 10 nM to 10uM dosing at a statistically significant level.

1110A **Parental dietary vitamin b12 causes intergenerational growth acceleration and protects offspring from microsporidian and bacterial pathogens** Winnie Zhao, Alexandra R Willis, Ronesh Sukhdeo, Aaron W ReinkeMolecular Genetics, University of Toronto

The parental environment of *C. elegans* can have a major impact on offspring fitness and immunity. Vitamin B12 is produced by some *Pseudomonas* bacteria that are encountered by *C. elegans* in the wild. This vitamin has previously been shown to accelerate the development of *C. elegans* and provide resistance to pathogenic *Pseudomonas aeruginosa*. We found that parents grown on diets supplemented with vitamin B12 or consisting of vitamin B12-producing bacteria produced progeny with accelerated growth. This inherited phenotype is vitamin B12 dose-dependent and persists for a single generation. During infection with *Nematocida parisii*, a natural microsporidian pathogen, the offspring of worms fed vitamin B12 diets have better reproductive fitness. However, no significant changes in pathogen load were observed. In addition, offspring of worms fed vitamin B12 diets are also resistant to killing by the bacterial pathogen *Pseudomonas vranovensis*. Using mutants of vitamin B12 pathways, we show that both intergenerational growth acceleration and microsporidia infection tolerance requires the methionine biosynthesis pathway. Protection from *P. vranovensis* killing depends on both the methionine biosynthesis pathway and the propionyl-CoA breakdown pathway. Together, our study demonstrates that bacteria can have both immune and dietary effects that are transmitted across generations which can influence infection and fitness outcomes of offspring.

1111A **Dietary bacteria regulate *C. elegans* lipid metabolism through Lysosome-related organelles** Shao-Fu Nien[1], Hsiao-Fen Han[1], Erh-Ya Lin[1], Yi-Cen Xie[1], Yu-Chia Lu[1], Chia-Hsuan Chang[1], Chao-Wen Wang[2], Yi-Chun Wu[1,3]1Institute of Molecular and Cellular Biology, National Taiwan University, [2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, [3]Institute of Atomic and Molecular Sciences, Academia Sinica

The maintenance of cellular organelle dynamics is critical for metabolic homeostasis and overall organismal health. While the influence of microorganisms and their metabolites on host physiology is well-established, the impact of microorganisms on organelle dynamics and their effects on host metabolism remain unclear. In our study, we used *C. elegans* and two different dietary bacteria, *Escherichia coli* (OP) and *Comamonas aquatica* (DA), to investigate the interspecies relationship. By RNAseq analysis, we found that bacteria DA differentially regulated lysosomal gene expression in the animals. Combining LysoTracker Red staining, fluorescent marker proteins, and Transmission electron microscopy (TEM) approaches, we demonstrated that DA promotes the biogenesis of lysosome-related organelles (LROs) in the intestine and its involved metabolites. Moreover, we employed LROs-deficient mutants and pulse-chase assays of BODIPY-C12 to dissect the function of LROs in response to different diets. The results revealed that LROs are fatty acid storage organelles that participate in DA-mediated lipid reduction. We also identified an acid lipase expressed in LROs that appear to hydrolyze specific TAG species and function to affect lipid metabolism. Altogether, our data revealed that the microorganism DA elicits responses on LRO biogenesis and that LROs may play a signaling role that affects lipid metabolism in response to microorganisms.

1112A **The role of MTCH2/MTCH-1 in lipid homeostasis, fertility and aging** Melissa Boldridge[1], Richard Ikegami[1], Anders M Naar[2], Veerle Rottiers[1,1]1UC Berkeley, [2]NST, UC Berkeley

Obesity has grown to epidemic proportions with an estimated four million deaths worldwide yearly caused by obesity associated-heart disease, -type 2 diabetes, -cancers and -COVID19 mortality. Apart from environmental factors, genetic predisposition plays a major role in obesity development as indicated by the discovery of many GWAS obesity genes. Here, we focus our studies on the role of *mtch-1,* the *C. elegans* homolog of MTCH2, a human GWAS obesity gene.

Through experiments in *C. elegans*, mammalian cell culture and mice, we found that MTCH2/*mtch-1* is a conserved regulator of lipid homeostasis, both required and sufficient to drive fat storage: MTCH2/*mtch-1* encodes an member of the SLC25 mitochondrial transporter family. Using a CRISPR Neongreen Knock-in line, we find that *mtch-1* is expressed ubiquitously on the mitochondrial outer membrane (MOM), not the inner mitochondrial membrane as are most SLC25 members. Apart from the lipid phenotypes, *mtch-1* depletion also extends lifespan (both in the mutant and by RNAi) and causes infertility . We find that *mtch-1* depletion activates the mito-UPR reporter (as measured by *hsp-6::gfp* activation), causes oxidative stress (as measured by activation of *gst-4::gfp*)and reduces oxygen consumption rate (as measured by Seahorse assay). To assess which tissues are important for which phenotypes and in an attempt to separate the lipid phenotypes from other phenotypes, we have generated a series of integrated tissues specific *mtch-1* expression lines with a range of expression levels. We are currently analyzing the lipid, aging and fertility phenotypes of these lines.

A recent study in mammalian cells claims that MTCH2 is a MOM protein insertase. Such a role in inserting many proteins in the MOM would explain the pleiotropic phenotypes caused by MTCH2/*mtch-1* and focusses our efforts to find interacting partners relevant for lipid homeostasis.

1113A **The hormones and neuronal circuit that mediate the gut-to-brain longevity signal.** Ling-Hsuan Sun[1], Jing-Cih Lin[2], Tsui-Ting Ching[2], Ao-Lin Hsu[3,41]National Yang Ming Chiao Tung University, [2]Biopharmaceutical Sciences, National Yang Ming Chiao Tung University, [3]Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [4]Internal Medicine, University of Michigan

Dietary restriction (DR) is a intervention that robustly delays aging and increases healthy lifespan in a wide variety of species. In previous studies, *sams-1*, which encodes an S-adenosyl methionine synthetase, has been identified as a key mediator in DR-induced lifespan extension. In this study, we have identified *ins-29* and *nlp-12* to be the gut-initiated hormonal signals that mediate the increased longevity in DR animals. In *sams-1* mutants and DR animals, the expression of *ins-29* is suppressed, whereas the expression of *nlp-12* is significantly upregulated. INS-29, an insulin-like peptide, is mainly expressed in neurons and the posterior intestinal cells. Intriguingly, we found that NLP-12, a neuron peptide, is expressed only in the tail interneuron DVA. Furthermore, we found that intestine-specific knockdown of *ins-29* is sufficient to upregulate NLP-12 expression in DVA neuron and extend lifespan. Furthermore, it has been reported that NLP-12 may serve as the ligand for *ckr-1* or *ckr-2,* the worm homologs of the mammalian Cholecystokinin receptors. Our epistasis analysis suggested that expression of *ckr-2* in a small subset of neurons may be required for the DR-induced longevity mediated by the *sams-1-ins-29-nlp-12* axis. Together, we have identified a novel gut to neuron signaling circuit that might be responsible for the cell-nonautonomous regulation of DR-induced longevity in *C. elegans*

1114A **Neuronal H3K4 methylation is essential for activation of the mitochondrial Unfolded Protein Response** Yoke Fei Pang, Alan Whitmarsh, Gino PoulinUniversity of Manchester

An important stress response associated with ageing and disease is the mitochondrial Unfolded Protein Response (UPRmt). Induction of the UPRmt leads to metabolic remodelling as well as increased expression of protective chaperones and proteases. Since the UPRmt is essentially a transcriptional response, we explored whether a chromatin modification associated with active transcription, methylation at histone 3 lysine 4 (H3K4me), is involved. To block all degrees of H3K4me, we used a null allele for *rbbp-5.* RBBP-5 is a core component essential to nucleate the H3K4 methyltransferase complex and enable its docking to the nucleosome. Using spike-in ChIP-seq, we show that H3K4me1/me2/me3 levels are almost completely depleted in the absence of RBBP-5. To assess the role of RBBP-5 in the UPRmt, we used the characterised *hsp-6::gfp* reporter strain which displays a robust increase in expression following mitochondrial stress. We found that in the absence of RBBP-5, *hsp-6::gfp* induction is strikingly reduced, indicating that RBBP-5 is required for the induction of the UPRmt. To verify whether this effect was specific for the UPRmt, we showed that RBBP-5 was not required for the UPR in the endoplasmic reticulum or for the heat shock response. We additionally tested the mitochondrial bioenergetic stress response and revealed it is constitutively activated in the absence of RBBP-5, suggesting RBBP-5 is important in the supply of energy. Finally, we tested whether over-expression of RBBP-5 in neurons could restore activation of the UPRmt in other tissues lacking RBBP-5. Neurons are the main proximal tissue that regulates, via mitokines, the induction of the UPRmt in distal tissues. Indeed, neuronal restricted expression of RBBP-5 is sufficient to restore activation of the UPRmt in the intestine when a mitochondrial stress is applied. This result implies that the presence of RBBP-5 in the intestine is not required for induction of the UPRmt there. Supporting this, another phenotype of the UPRmt, nuclear shrinkage, was unaffected in the absence of intestinal RBBP-5. We are currently analysing whether a key transcriptional regulator of the UPRmt, ATFS-1, accumulates normally in gut nuclei of *rbbp-5(-)*mutants. Taken together, these results show that neuronal RBBP-5 and the associated H3K4 methylation play a specific role in the systemic activation of the UPRmt.

1115A **Exploring the link between coregulatory protein SIN-3, mitochondrial homeostasis and metabolism** Marina Giovannetti[1], Ophelie Nicolle[2], Gregoire Michaux[2], Marta Artal Sanz[3], Michael Witting[4], Francesca Palladino[51]ENS Paris, [2]Rennes University, [3]Andalusian Centre for Developmental Biology, [4]Helmholtz Zentrum, [5]LBMC, Ecole Normale Supérieure de Lyon, Lyon University

The SIN3 transcriptional coregulator influences gene expression through multiple interactions that include histone deacetylases (HDACs). Mammalian SIN3 plays a key role in cancer progression, and haploinsufficiency and mutations in SIN3 are the underlying cause of Witteveen-Kolk syndrome and related intellectual disability (ID)/autism syndromes, emphasizing its important regulatory role. Depletion experiments in various species have shown that SIN3 proteins play a role in the maintenance of functional mitochondria through unknown mechanisms. In *C. elegans,* knock-down of the single SIN3 homologue *sin-3* was shown to result in a decreased lifespan accompanied by hypopolarisation of mitochondrial membrane potential, enhanced autophagy and increased oxidative stress [1,2]. The underlying cause of these defects, and whether they are associated with changes in mitochondrial homeostasis and metabolism was not investigated. Here, using a CRISPR-Cas9 null allele, we show that loss of SIN-3 results in deregulation of mitochondrially encoded genes and nuclear-encoded mitochondrial genes. Mitochondrial UPR (UPR^{mt}), a transcriptional response to mitochondrial stress, is dampened in the absence of SIN-3. *in vivo* imaging and transmission electron microscopy (TEM) revealed severe mitochondrial fragmentation in all tissues analyzed, including muscle, hypodermis and intestine. Consistent with a defect in mitochondrial function, as *sin-3* mutants age, both basal and maximal respiration (OCR, oxygen consumption rate) dramatically increases, and spare capacity is reduced compared to wild type animals at the same stage. Using metabolomic analysis, we identify signatures of mitochondria stress, and deregulation of methionine flux resulting in decreased levels of S-adenosyl-methionine (SAM) and increased spermidine. These correlate with changes in the expression of key enzymes in these pathways. We propose that SIN-3 may provide a link between chromatin-based regulatory mechanisms, mitochondrial function and metabolism.

Pandey, R.; *et al. Aging* **2018**, *10*, 3910–3937

Sharma, M. *et al. Autophagy* **2018**, *14*, 1239–1255

1116A **Phenotypic expansion of *ATP5F1A* supported with functional studies of dominant missense variants associated with intellectual disability and developmental delay not previously observed with recessive variants** Sara M Fielder[1], Tara E Samson[1], Jian Chen[2], Weimin Yuan[1], Jacob Lesinski[1], Hieu Hoang[1], Jill A Rosenfield[3], Lisa Emrick[3], Lindsay Burrage[3], Kim C Worley[3], William Craigen[3], Cecelia Esteves[4], Kayla Treat[5], Erin Conboy[5], Francesco Vetrini[5], Dustin Baldridge[1], Gary A Silverman[1], Tim Schedl[2], Stephen C Pak[11]Pediatrics, Washington University School of Medicine, [2]Washington University School of Medicine, [3]Molecular and Human Genetics, Baylor College of Medicine, [4]Harvard Medical School, [5]Undiagnosed Rare Disease Clinic, Indiana University School of Medicine

ATP5F1A encodes the alpha subunit of ATP synthase and has previously been associated with Mitochondrial Complex V Deficiency (MC5DN4) and Combined Oxidative Phosphorylation Deficiency 22 (COXPD22). Both diseases are recessive, present with encephalopathy and seizures, and are fatal within the first two years of life. Here, we describe three probands, two identified through the Undiagnosed Diseases Network and one at the Undiagnosed Rare Disease Clinic at the Indiana University School of Medicine, with intellectual disability and global developmental delay that have heterozygous, *de novo* missense variants of unknown significance in the nuclear *ATP5F1A* gene. All three probands have features that are distinct from previously reported recessive cases in that: 1) the probands are heterozygous, 2) they are all over the age of two (two are teenagers), and 3) none of the probands reported having seizures. Recently, other cases of *de novo* missense variants in *ATP5F1A* were identified in a cohort of 2,962 patients diagnosed with mitochondrial disease and/or dystonia, expanding the spectrum of ATPase-related disorders, however, *in vivo* functional studies were not performed. To determine if our proband variants are damaging to protein function *in vivo*, we modeled all three in *C. elegans* by CRISPR/Cas9-mediated editing of the orthologous gene, *atp-1.* All three variants are homozygous L1 larval lethal, similar to *atp-1* deletion homozygotes. Heterozygotes for the three variant-edited animals were small, slow growing, and infertile, indicating that the variants were all damaging to *atp-1* function. Given that the *atp-1* null heterozygotes were superficially normal and fertile, the proband variants are likely to be gain-of-function and not haplo-insufficient. Moreover, infertility of heterozygous animals was rescued by additional wild type copies of *atp-1*, indicating the variants are likely dominant-negative poisons. Animals heterozygous for the two variants analyzed showed mitochondrial stress as determined by the activation of the mitochondrial unfolded protein response reporter (hsp-6::GFP), suggesting that the variants reduce ATP synthase function. We conclude that the three *atp-1* variants are damaging to ATP synthase complex function in *C. elegans*. By extension, the three proband variants are likely damaging to *ATP5F1A* function, are likely responsible for the dominant phenotypes seen in the probands, and thus expand the genetic mechanisms of *ATP5F1A* disease.

1117A **Base Excision Repair drives age-related neurodegeneration in Parkinson's disease models** Francisco Jose Naranjo Galindo[1,2], Tanima Sengupta[1], Konstantinos Palikaras[3,4], Qin Ying Esbensen[1,5], Georgios Konstantinidis[4], Kavya Achanta[6], Henok Kassahun[1], Ioanna Stavgiannoudaki[4], Vilhelm A. Bohr[6,7], Mansour Akbari[8], Nektarios Tavernarakis[4,9], Nicola Pietro Montaldo[1], Hilde Nilsen[1,2,51]University of Oslo, [2]Oslo University Hospitals, [3]University of Athens, [4]Institute of Molecular Biology and Biotechnology (IMBB), [5]Akershus University Hospital, [6]University of Copenhagen, [7]National Institute on Aging, [8]UiT- The Arctic University of Norway, [9]University of Crete

Genomic instability is one of the hallmarks of ageing and a risk factor in the development of diverse neurodegenerative diseases (NDD) such as Parkinson's disease (PD). Different DNA repair mechanisms targeting different types of DNA lesions counteracts genomic instability. One of these mechanisms is the Base Excision Repair (BER) pathway, dedicated to the removal of single-base, non-helix distorting damage in nuclear and mitochondrial DNA where, first, a DNA glycosylase detects the damage, removes the damaged nucleotide and, in some cases, also creates a single strand break (SSB). In case the DNA-glycosylase cannot create the SSB, that function is fulfilled by an AP endonuclease. This is followed by the action of a DNA polymerase and a DNA ligase that synthetizes and seals in the new base, respectively. While in mammals there is a wide overlap of functions between the different components of this pathway, in *Caenorhabditis elegans* (*C. elegans*), BER is simplified, serving as a great model for the understanding of the isolated function of each of its components. Here we show that, in *C. elegans* PD model, the lack of the initial damage-recognition step, carried out by the NTH-1 DNA glycosylase, does not cause a deficit in healthspan, but in contrast, is neuroprotective. The dopaminergic neurons of animals expressing α-synuclein and with a deficiency of NTH-1, remain morphologically fitter than the isogenic NTH-1 proficient strain and, notably, with improved neuronal functionality. Besides the expected impact in the genomic instability, a dysfunction in the energetic processes should be expected, due to the function of NTH-1 in mitochondria. This should be especially problematic in a highly metabolic tissue as the neuronal circuit. On the contrary, NTH-1 deficiency increases the mitochondrial transcription rate, activating mitohormesis, the source of neuroprotection in our model. This effect disappears when re-supplementing NTH-1 from an extrachromosomal array. In addition, expressing the human homologue of NTH-1, NTHL1, in the dopaminergic circuit, also ablates neuroprotection. Similarly, the neuroprotection is restored when exposing these humanized nematodes to NTHL1 inhibitors. This not only highlights the possibilities of the organism for the study of human BER as a vacuumed environment with much less overlap of function between the enzymes of this pathway, but also points at DNA-glycosylases as potential targets in the treatment of NDD.

1118A **An Ortholist-RNAi screen identifies new transcription factors and epigenetic regulators of the mitochondrial unfolded protein response upon prohibitin depletion** Jesus Fernandez-Abascal, Blanca Hernando-Rodriguez, Maria Jesus Rodriguez-Palero, Marta Artal-SanzDepartment of Molecular Biology and Biochemical Engineering, Andalusian Centre for Developmental Biology, Consejo Superior de Investigaciones Científicas/Junta de Andalucía/Universidad Pablo de Olavide

Aging is characterized by physiological decline and increased risk of age-related diseases. The mitochondrial prohibitin (PHB) complex, a ring-like structure in the inner mitochondrial membrane, is critical to mitochondrial function and proteostasis. Depletion of PHB has opposite effects on aging, shortening lifespan in wild-type worms while extending the lifespan of insulin/IGF-1 signaling (IIS) pathway mutants. Lack of PHB strongly induces the mitochondrial unfolded protein response (UPR^{mt}) to maintain mitochondrial proteostasis, while the UPR^{mt} is attenuated in IIS *daf-2* mutants upon PHB depletion. In this study, we aimed at identifying new pathways involved in the regulation of the PHB-mediated mitochondrial stress response, as well as mechanisms responsible for the opposite longevity outcomes of PHB depletion. Towards this aim, we carried out a genome-wide RNAi screen for *C. elegans* genes having a human orthologue in PHB-depleted wild type animals and PHB-depleted IIS mutants. We uncovered both known and new PHB genetic interactors affecting the UPR^{mt} in the different genetic backgrounds. We identified two new transcription factors as specific regulators of the mitochondrial stress response. We further established chromatin remodeling via histone deubiquitination as a strong differential modulator of the mitochondrial stress response and aging upon PHB depletion in wild type and IIS mutants. Overall, this study identifies new players specifically involved in the regulation of the mitochondrial stress response and longevity in an IIS dependent manner and sheds light on the processes contributing to the differential effect in aging of PHB depletion in wild type and metabolically compromised animals.

1119A **The DREAM complex regulates DNA repair capacity in somatic tissues** Arturo Bujarrabal[1,2], Georg Sendtner[1,2], David H Meyer[1,2], Georgia Chatzinikolaou[3], Kalliopi Stratigi[3], George A Garinis[3], Björn Schumacher[1,21]Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, [2]Cologne Excellence Cluster for Cellular Stress Responses in Ageing-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, [3]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Department of Biology, University of Crete

The DNA is constantly exposed to genotoxic insults that challenge organismal health maintenance. Different mechanisms survey the genome to ensure its integrity, but their efficiency differ depending on whether the cells are somatic or germ cells, as well as their proliferative and differentiation status. In germ cells, DNA repair is highly efficient and accurate, leading to low mutation rates compared to somatic cells. In addition, replicating cells can use high-fidelity repair mechanisms such as homologous recombination repair. To repair UV-lesions, dividing cells rely on global genome nucleotide excision repair, whereas quiescent cells mostly use transcription-coupled nucleotide excision repair. In *C. elegans*, repair pathways are highly conserved and can be easily studied in the context of postmitotic somatic cells and the highly proliferative germline.

The DREAM complex is a conserved transcriptional repressor of cell cycle genes that promotes quiescence. Using *C. elegans*, we found that DREAM can bind and repress multiple genes involved in the DNA damage response. A deficiency in this complex leads to a gene expression signature enriched with DNA repair genes that resembles the germline expression profile. DREAM complex

deficient mutants show a remarkable resistance and improved repair of various DNA-damage types, both during development and aging. The chemical inhibition of DREAM in human U2OS cells also boosts DNA repair gene expression and consequently increases DNA damage resistance. Furthermore, DREAM inhibition in progeroid *Ercc1*$^{-/-}$ mice prevents the loss of photoreceptors, a hallmark of the progeroid Cockayne Syndrome.

We show that the DREAM complex represses all major DNA repair pathways in somatic tissues, thus limiting their resistance to genotoxic stress in *C. elegans*. The chemical inhibition of DREAM in human cells and mice also increases genome stability. Thus, the DREAM complex functions as a conserved regulator of DNA repair capacity.

1120A **Characterizing the role of Protein Kinase G (PKG/EGL-4) in the hypoxia stress response** Tatiana Popovitchenko, Eun Chan Park, Christopher RongoWaksman Institute, Rutgers University

The hypoxia stress response is a coordinated process that allows for survival under low oxygen conditions. Low oxygen, or hypoxia, manifests from either environmental (*e.g.* hypoxic niches) or pathological (*e.g.* ischemic stroke) changes. The canonical pathway is ubiquitous and well conserved from nematodes to mammals. Under aerobic conditions, a prolyl hydroxylase (EGL-9) decorates the constitutively produced transcription factor HIF-1 with hydroxyl groups, which allows the E3 ubiquitin ligase VHL-1 to recognize and target HIF-1 for degradation. By contrast, hypoxic conditions inactivate EGL-9, resulting in HIF-1 stabilization, followed by changes in gene expression and metabolism. Indeed, our lab has recently profiled the systemic trancriptomic and metabolic response to activation of this pathway. Hypoxia and EGL-9 also regulate locomotory behavior through changes in the synaptic localization of GLR-1 glutamate receptors via a HIF-1-independent non-canonical version of the pathway. We previously revealed a novel regulator of the pathway, the p38 MAP Kinase (PMK-1). PMK-1 acts upstream of EGL-9, suggesting potential cross-regulation between the hypoxia response and other environmental signaling pathways. Through a phosphoproteomics screen, we have identified the Protein Kinase G (PKG) ortholog EGL-4 as a potential substrate of PMK-1. We tested for a role of EGL-4 in the hypoxia response and found a mild effect of *egl-4* mutations on the expression of transcriptional reporters for two known HIF-1 target genes. We also observed defects in GLR-1 synaptic localization and GLR-1-mediated behavior in *egl-4* mutants. Our results suggest that PKG/EGL-4 signaling modulates the hypoxia stress response, which increases our understanding of how the hypoxia response pathway is integrated with signaling systems that respond to other environmental cues.

1121A **Using forward genetic screens to probe the neuronal-mediated *C. elegans* immune response to oomycete exposure** Domenica Ippolito, Manish Grover, Mark Hintze, Ming Yi, Michalis Barkoulas Life Sciences, Imperial College London

Our lab has recently established a new pathosystem based on oomycetes found to naturally infect nematodes including *Caenorhabditis elegans.* We have previously reported that chemosensory neurons may mediate recognition of oomycetes to initiate a signalling cascade leading to the activation of a protective transcriptional program called the oomycete recognition response (ORR), a hallmark of which is the induction of *chitinase-like* (*chil*) genes in the epidermis that antagonize the infection. In order to elucidate the identity of the pathogen recognition pathway and the machinery involved in the cross-tissue comunication exchange between sensory neurons and the epidermis, we have performed forward genetic screens using induction of *chil-27::GFP* as the phenotypic readout and an innocuous pathogen extract that is sufficient to trigger the immune response without causing any infection. These screens have allowed us to retrieve two classes of mutants, namely "*chili*" (*chil* induction lacking infection), which exhibit oomycete-independent constitutive *chil-27* gene expression, and "*no chili*" (no *chil* induction) mutants, which show complete or partial loss of *chil-27* gene induction upon pathogen extract exposure. While *no chili* mutants have allowed us to uncover specific regulators of ORR functioning both in neurons and epidermis, *chili* mutants have revealed regulators of the immune response in the epidermis, which may be also regulating responses to other natural pathogens of *C. elegans.* We present here a summary of previous and ongoing screens aiming at reaching saturation in dissecting the composition of the pathogen recognition pathway, and circumventing issues with the mapping strain CB4856 partially modifying the phenotype. Defining the components of this defence pathway will allow us to increase our knowledge on pathogen recognition mechanisms in *C. elegans* and gain insights into oomycete recognition in animals more broadly.

1122A **Non-autonomous induction of the endoplasmic reticulum unfolded protein response by COL-75 missense variants** Hung-Jen Shih[1], Jeeyeon Cha[1], Stephane Flibotte[2], Patrick Hu[11]Medicine, Vanderbilt University Medical Center, [2]Life Sciences Institute, University of British Columbia

One third of all proteins are cotranslationally translocated into the endoplasmic reticulum (ER) during their 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 to accommodate changes in unfolded protein load. Disruption of ER homeostasis during the aging process can lead to ER luminal protein misfolding and aggregation, a condition known as "ER stress" which is associated with common human illnesses such as Alzheimer's disease, Parkinson's disease, and diabetes. Thus, understanding the molecular mechanisms that govern ER homeostasis is likely to illuminate the pathogenesis of aging-related

diseases. Whether or how physiologic ER stress in specific cells is communicated organismally is poorly understood.

While studying the function of the conserved ER translocon component TRAP-1/SSR1, 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/SSR1 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 (*dp691*) that causes constitutive *hsp-4::GFP* expression. Collagen biosynthesis is a highly choreographed process involving cotranslational ER translocation, multiple post-translational modifications, and triple helix formation. 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(dp691)* requires *xbp-1,* demonstrating that *col-75(dp691)* activates the canonical IRE-1/XBP-1 arm of the ER UPR. Surprisingly, while a transcriptional *col-75p::dsRed* reporter is expressed in the excretory cell, amphid and phasmid glia, and vulval muscles, *col-75* missense alleles induce *hsp-4::GFP* expression specifically in the intestine and spermatheca. We hypothesize that *col-75* missense variants induce *hsp-4::GFP* expression non-autonomously by influencing a signal secreted from *col-75*-expressing cells that conveys information about ER homeostasis to other tissues in the organism.

1123A **Nucleotide excision repair protects from endogenous and exogenous formaldehyde-induced DNA damage in *C. elegans*** Matthias Rieckher[1], Lucas B Pontel[2,3], Björn Schumacher[41]Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, and Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), [2]1.Josep Carreras Leukaemia Research Institute, [3]2.Biomedicine Research Institute of Buenos Aires (IBioBA-MPSP), Max Planck Partnership Insitute, [4]Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, and Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), and Center for Molecular Medicine Cologne (CMMC)

Formaldehyde (FA) is a potent environmental and endogenous toxin that causes a variety of DNA damage, including crosslinks, DNA double strand breaks and base modifications. The loss of the FA-detoxifying alcohol dehydrogenase 5 (ADH5) causes progeroid phenotypes in mouse models for Cockayne Syndrome and Fanconi Anemia, respectively, while inherited mutations in the genes coding for ADH5 and aldehyde dehydrogenase 2 (ALDH2) enzymes result in inherited bone marrow failure syndrome (IBMFS) in humans, which is corroborated in transgenic mice. While these models demonstrate detrimental effects of endogenous FA, the precise molecular toxicity mechanisms have yet to be revealed. We characterized the FA sensitivity of numerous *C. elegans* DNA repair deficient strains and found increased somatic sensitivity in mutants for the Nucleotide Excision Repair (NER). Upon FA exposure, simultaneous loss of *C. elegans* ADH-5 and Transcription-coupled (TC)-NER causes embryonic lethality, while AHD-5 and Global genome (GG)-NER double-mutants show elevated developmental delay. Curiously, loss of ADH-5 and XPA-1, which acts downstream of TC-NER and GG-NER, causes severely decreased fecundity even without FA exposure. Developmental defects and sterility in single and double mutants for ADH-5 and NER were exacerbated upon downregulating *C. elegans* ALDH2 (*alh-1*), resulting in reduced baseline fertility and somatic development, and severely reduced FA tolerance. Previously, we had demonstrated that FA cytotoxicity partially derives from scavenging glutathione (GSH), thereby causing substantial cellular ROS accumulation. Indeed, fecundity and developmental defects in mutants with simultaneous loss of aldehyde-detoxification and NER are partially suppressed upon treatment with the GSH precursor N-acetyl-l-cysteine (NAC). Our results establish genetic *C. elegans* models to study endogenous FA-toxicity, which will aid in understanding the molecular underpinnings of progeroid diseases and reveal potential therapeutic interventions.

1124A **Modelling human diseases in *C. elegans*: facts and challenges** Julián Cerón[1], Dmytro Kukhtar[2], Guillem Escalona[2], Victor Muñoz-Villena[2], Jeremy Vicencio[2], Carmen Martínez-Fernández[2], Xènia Serrat[2], Alba Olaso[21]Modelling Human Diseases in C. elegans, Bellvitge Biomedical Research Institute - IDIBELL, [2]Bellvitge Biomedical Research Institute - IDIBELL

C. elegans researchers working in biomedical institutions can suffer the impostor syndrome to justify their studies in a scientific environment oriented towards the clinic. The fact that findings in *C. elegans* are accelerating other discoveries may not sound appealing enough to certain people evaluating and funding our work. Nobel Prizes, or key discoveries such as RNAi and the apoptotic pathway, are solid shields against criticisms but we need to invest in developing better weapons to fight in the field of translational research.

Our lab, located within a hospital, is interested in developing tools and strategies related to CRISPR-Cas and the humanization of *C. elegans* genes to improve our capacity to model human diseases. We validated the use of minimal PAM Cas9 nucleases (SpG and SpRY) in animals, showing that these engineered nucleases are efficient and specific to edit genomes using NGN and NAN as PAM sequences. Thus, we can target specific sequences as microRNAs that may not contain an NGG PAM, and increase the efficiency of genome editing by improving the proximity of the Cas9 cut to the edit site. Such proximity is relevant when mimicking diseases caused by missense mutations. Also related to CRISPR, we developed the Nested CRISPR approach, which is an efficient strategy for precise insertions of long DNA fragments in endogenous loci.

The humanization of a *C. elegans* gene may help to evaluate the functional impact of gene variants and mutations. It could be helpful to determine the impact of mutating a residue important for protein structure, and also to study sensitivity to certain drugs. In any case, it is essential to prove that humanized proteins are fully functional, and replacing the gene at its endogenous locus helps to express the humanized protein at the right physiological levels. The humanization of proteins with multiple protein-protein interactions would be particularly challenging as exemplified by the splicing factor *prp-3*, which tolerates partial humanization only. However, such chimeric protein, part human-part nematode, would be of interest to address specific questions. As an example, we humanized a region of *sftb-1* that is important for small molecule binding. By doing so, we made *C. elegans* sensitive to splicing modulators and consequently suitable to investigate the response to these compounds and synergies with other small molecules.

In this context, we will comment on our projects related to Retinitis Pigmentosa, Menkes disease, and cancer to exploit *C. elegans* in the investigation of human diseases.

1125A **Notch signaling in germline stem cells controls reproductive aging in *C. elegans*** Aaron M Anderson[1], Zuzana Kocsisova[1,2], Tim Schedl[2], Kerry Kornfeld[11]Department of Developmental Biology, Washington University School of Medicine, [2]Department of Genetics, Washington University School of Medicine

Adult stem cell exhaustion is a hallmark of aging since an age-related decline of these cells is implicated in degenerative changes of tissue structure and function. However, mechanisms of stem cell exhaustion are poorly understood. To model this process, we are characterizing the causes of age-related changes in the *Caenorhabditis elegans* germline stem cell system. Using immunostaining for mitotic germ cells in a time course of aging adult hermaphrodites, we observed rapid and pervasive declines in the number, cell cycle rate, and meiotic entry rate of germline stem cells by adult days 3 and 5. These changes are well-correlated with the progressive decline in hermaphrodite fertility, indicating that exhaustion of adult stem cells in the germline is a major cause of reproductive aging in worms. By immunostaining for reporters of Notch ligand (LAG-2) and effectors (LST-1 and SYGL-1), we found similar declines in the stem cell niche and intrinsic Notch signaling activity, respectively. Ectopically expressing SYGL-1 in the germline delayed age-related changes in stem cell number and activity. This suggests that Notch signaling between the stem cell niche and germ cells decreases with age, and increased Notch activity in the germline stem cells is sufficient to sustain them. These data support the idea that adult stem cell exhaustion in the germline is an origin of the reproductive aging in hermaphrodites, and the germline stem cells decline due to a failure in maintenance by the niche.

1126B **Photoconvertible fluorescent protein-tagged tau exhibits exceptional stability in a *C. elegans* model of tau proteostasis** Marina Han[1], Aleen Saxton[2], Heather Currey[2], Nicole Liachko[2,3], Sarah Waldherr[2], Brian Kraemer[2,3,4]1University of Washington Graduate Program in Neuroscience, [2]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, [3]Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, [4]Department of Psychiatry and Behavioral Sciences, University of Washington

A predominant theme in neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, ALS, and Huntington's disease, is aging dependent pathological protein aggregation of specific proteins unique to each disease, collectively referred to as proteinopathies. Our research utilizes the abundant advantages of working with *C. elegans* to explore the mechanisms of several proteinopathies and their relationships with aging, behavior, and neuronal health. Here, we present a recently generated series of transgenic models of pathological tau, a hallmark proteinopathy occurring in Alzheimer's disease and Frontotemporal Lobar Degeneration (FTLD-tau). We constructed strains carrying integrated multicopy or single copy transgenes expressing Dendra2-tagged human tau under control of the pan-neuronal *snb-1* promoter. High level expression of human tau in *C. elegans* neurons has previously been shown to cause significant motor deficits, shortened lifespan, and increased neurodegeneration as measured by loss of GABAergic motor neurons. FTLD-causative mutations in the human tau encoding gene, cause similar phenotypes at much lower levels of tau expression than wild type tau. While previous models of tauopathy provide clear insights into disease mechanisms, untagged tau cannot be monitored in living animals. Tau tagged with Dendra2 photoconvertible fluorescent protein allows for real-time *in vivo* tracking of protein synthesis, localization, processing, and degradation. To understand the impact of tau concentration on its proteostatic properties, we generated a series of multicopy lines at varying expression levels by traditional UV integration of extrachromosomal arrays and a series of single copy lines utilizing recombinase-mediated cassette exchange (RMCE) at established landing sites. Initial evaluation showed that single copy strains express similar levels of Dendra2::tau from distinct genomic insertion sites, all at low levels. In contrast, multicopy lines exhibited a wide range of Dendra2::tau expression levels. Neuronal expression of the N-terminally tagged Dendra2::tau protein produced similar behavioral deficits to existing untagged tau expressing models with tau expression level correlating with phenotype severity. Preliminary pulse-chase experiments, used to assess tau turnover, in multicopy strains reveal a neuronally expressed tau half-life that is longer than the median lifespan of *C. elegans*. While single-copy Dendra2::tau strains lack distinguishable locomotion deficits due to very low tau levels, they may have utility to identify new enhancers of tau accumulation, as evidenced by phenotype exacerbation with human wild-type TDP-43 overexpression. In summary, we present single- and multicopy Dendra2::tau models as a

novel tool to study tau proteostasis and modifiers of tauopathy.

1127B **The death of lifespan: Using movement to measure ageing** Giulia Zavagno[1], Adelaide Raimundo[2], Christopher Saunter[2], David Weinkove[1,21]Department of Biosciences, Durham University, [2]Magnitude Biosciences Ltd

With a lifespan of 2-3 weeks, *C. elegans* offers a rapid and powerful *in vivo* method to determine whether a compound slows ageing. However, measuring lifespan produces only one datapoint per animal – the time of death. Manual lifespan assays have been used for decades because of the relative ease and transferability of the assay. Here we describe an alternative to manual or automated lifespan that measures ageing by automated monitoring of movement from early to mid-adulthood. At 24°C, most functional decline occurs during the first week of *C. elegans* adulthood. Our unique technology, The Wormgazer™, can image over 100 petri dishes simultaneously and non-invasively, using an array of cameras and automated analysis. This approach reveals a large amount of data about the movement of animals through their active life. We show that the long-lived mutant *age-1(hx546)* stays active for longer than the wild type but moves slower in early adulthood. We find that 7 days of imaging is sufficient to detect interventions that slow ageing. For example, the dose-dependent age-slowing effect of sulfamethoxazole could be detected in 7 days while a parallel lifespan experiment took 40 days to complete. Thus, detecting changes in movement with automated imaging provides a fast, robust method to screen compounds for efficacy to slow ageing, measuring an endpoint of intense human interest: the continuation of health with age. With technologies that can detect changes in movement, lifespan analysis is no longer necessary.

1128B **The loss of gap junctions in excitable cells promotes mitochondrial stress-induced longevity in *C. elegans*** Daniel-Cosmin Marcu[1], Nathalie Alexandra Vladis[2], Katharina Elisabeth Fischer[3], Roderick N. Carter[4], Peter Askjaer[5], Nicholas M. Morton[4], Karl Emanuel Busch[11]Institute for Mind, Brain and Behavior, Faculty of Medicine, HMU Health and Medical University, Potsdam, [2]Department of Biology, Brandeis University, [3]Centre for Discovery Brain Sciences, University of Edinburgh, [4]University/British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, [5]Andalusian Center for Developmental Biology (Consejo Superior de Investigaciones Científicas, Universidad Pablo de Olavide

The nervous system is a central regulator of longevity, but how communication between excitable cells affects ageing and lifespan is poorly understood. We investigated whether gap junctions play a role in regulating longevity and ageing, and found that mutants of the innexin genes that encode gap junction proteins in *C. elegans* have extensive and diverse effects on lifespan. Loss of the innexin *unc-9* from either the nervous system or muscles, for example, increases lifespan by a third, and also improves healthspan. The extension of lifespan in *unc-9* mutants requires reactive oxygen species. Loss of *unc-9* increases mitochondrial respiration and triggers a systemic induction of the mitochondrial-specific unfolded protein response (UPR^{mt}) to promote longevity. Thus, gap junctions in excitable cells can modulate ageing and lifespan through their effect on mitochondrial respiration.

1129B **Coelomocytes activate by mobilizing and ramifying in response to alcohol** Jon Pierce[1], Zheng Wu[1], Andy Cardona[1], Ben Clites[1], Alyssa Marron[1], Brooke Frohock[1], Erik Anderson[21]The University of Texas at Austin, [2]Northwestern University

C. elegans has been a useful model for studying behavioral responses to alcohol with relevance to Alcohol Use Disorder in patients. Through a GWAS study on acute ethanol sensitivity in *C. elegans*, we identified a deleterious variant in *lgc-24* that was associated with hypersensitivity to intoxication. LGC-24 is an orphan ligand-gated ion channel in the cys-loop family. *lgc-24* is exclusively expressed in coelomocytes. To determine if coelomocytes respond to ethanol, we observed them with a RFP reporter. We found that coelomocytes in untreated control N2 worms are ovoid cells that are anchored to the body wall in three pairs. Upon ethanol treatment, however, within 20 minutes most coelomocytes lose anchor and acquire a highly dynamic shape that changes along with movement of the worm. In this process, coelomocytes "activate" akin to macrophages and microglia. Often several large vesicles bleb off and drift freely in the body cavity fluid. Long (50-300 micrometer) nanofilament branches and/or thicker pseudopodia sprout, some which stretch past the pharynx into the nerve ring. In the later stage of activation, RFP tubular networks are off loaded in the hypodermis, which resemble the previously-reported tubular lysosomes. In addition to ethanol treatment, laser damage to head neurons and expression of human disease proteins also trigger localized coelomocyte activation. The highly variable morphology in activated coelomocytes suggests that they may have unknown roles besides serving as stationary scavenger cells.

To study the role of LGC-24 in coelomocyte activation, we investigated coelomocytes in *lgc-24* mutants. We found that coelomocytes in three independent *lgc-24* loss-of-function mutants displayed constitutively activate morphologies even in healthy untreated larvae. Rescue of *lgc-24(+)* in these mutants restored both normal ethanol sensitivity and coelomocyte morphology. Conversely, overexpression of LGC-24 appeared to suppress coelomocyte activation. LGC-24 is homologous to acetylcholine receptor subunits. The cholinergic anti-inflammatory pathway is important for the regulation of macrophage and microglia activation, our results suggest that this pathway might be conserved in *C. elegans*.

In conclusion, coelomocytes can be rapidly activated into highly dynamic cells by ethanol, and the cys-loop ligand-gated ion channel LGC-24 appears to repress activation. Our study on ethanol-activated coelomocytes will help establish *C. elegans* as a model organism in alcohol and immune response.

1130B **SAM deficiency in the mitochondria induces longevity through mitochondrial unfolded protein response** Feng-Yung Wang[1], Tsu Yu Chen[2], Pin-Jun Lee[2], Ao-Lin Hsu[1,3], Tsui-Ting Ching[41]Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [2]Institute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University, [3]4Department of Internal Medicine, Division of Geriatric and Palliative Medicine, University of Michigan, [4]INstitute of Biopharmaceutical Sciences, National Yang Ming Chiao Tung University

S-adenosyl methionine synthetase-1(SAMS-1) is the main enzyme generating S-adenosyl methionine (SAM), the universal methyl group donor, in *C. elegans.* Previous studies have indicated that RNAi silencing or knockout of *sams-1* could induce a robust mitochondrial unfolded protein response (UPR^{mt}) and lifespan extension in *C. elegans*. Our study found that downregulating SAMS-1 markedly decreases mitochondrial SAM levels. Moreover, RNAi knockdown of SLC-25A26, a carrier protein responsible for transporting SAM from the cytoplasm into the mitochondria, could significantly diminish SAM levels in mitochondria and activate UPR^{mt}, suggesting that *sams-1*-mediated UPR^{mt} activation may depend on mitochondrial SAM levels. Through a mitochondrial methyltransferase RNAi screen, we identified C56G2.3, a mitochondrial tRNA methyltransferase, as a major downstream effector of *sams-1* to regulate UPR^{mt} in *C. elegans*. Our findings indicate that lowering mitochondrial SAM levels by downregulating SAMS-1 impairs protein translation in mitochondria, consequently triggering UPR^{mt}. Furthermore, SAM-deficiency-induced UPR^{mt} contributes to lifespan extension in *sams-1* mutants.

1131B **Intestine-specific mitochondrial stress response protects against alcohol-mediated movement impairment** Annette Truchan, Hongkyun Kim Chicago Medical School, Rosalind Franklin University

Chronic, excessive alcohol use causes regional structural brain damage and cognitive disorders. Chronic alcohol misuse is also associated with a wide array of movement impairments. While alcohol-induced brain damage has been explained by alcohol's effects on neural excitability or nutritional deficiency, we do not fully understand the pathogenic mechanism at the molecular and cellular levels by which alcohol exerts its toxicity and damages the nervous system. The nematode *C. elegans* is an amenable model organism that can be used for dissecting the pathological mechanism of alcoholic neurotoxicity. We previously showed that while alcohol strongly induces many conserved cellular stress responses in *C. elegans*, its main toxic effects are centered on mitochondrial function. Moreover, we uncovered that wild-type animals exposed to a low dose of alcohol for 24 h exhibit greatly reduced swimming behavior in alcohol-free liquid media. Remarkably, we found that perpetual mitochondrial unfolded protein response (UPR^{mt}) spares from a motor function deficit caused by chronic alcohol exposure. Moreover, intestine-specific, but not neuron- or muscle-specific, UPR^{mt} activation with a single copy expression of the *atfs-1(gf)* gene is sufficient to protect against alcohol-mediated movement impairment. We hypothesize that activation of UPR^{mt} in the intestine generates a protective signal that delivers to neurons, which in turn protects against alcohol-mediated movement impairment. We are currently testing existing mutants to unravel genetic components that comprise this gut-to-brain axis. A detailed analysis of alcohol-mediated UPR^{mt} and its intra- and intercellular signaling in the context of alcohol-mediated movement impairment will lead to effective druggable targets that protect against, or ameliorate, alcoholic movement impairment.

1132B **Vitamin A extends lifespan in *Caenorhabditis elegans* via DAF-16/FOXO and SKN-1/Nrf2 pathways-mediated reduction of oxidative stress** Chaweewan Sirakawin[1], Dongfa Lin[1], Andre Pires-daSilva[2], Jingjing Wang[3], Ilya A. Vinnikov[11]Laboratory of Molecular neurobiology, Sheng Yushou center of Cell Biology and Immunology, Department of Genetics and Developmental Biology, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, [2]University of Warwick, [3]Shanghai Key Laboratory of Pancreatic Diseases, School of Medicine, Shanghai Jiao Tong University

Vitamin A (VA) is a micronutrient essential for survival and growth in many organisms, but its role in aging and age-related diseases remains unclear. In this work, we investigated how VA affects lifespan, oxidative stress, fat accumulation, stress resistance and gene expression in the nematode *Caenorhabditis elegans*, a well-established model for aging research. VA extended lifespan and reduced lipofuscin and fat accumulation by about 35-45% while increasing resistance to oxidative and heat stress. RNA-Seq analysis revealed that VA upregulated DAF-16/FOXO and SKN-1/Nrf2 pathways which are strongly implicated in regulation of aging and stress response. Using genetic and RNA interference models, we confirmed the causative interactions between VA-induced activation of these pathways and lifespan extension. Our study provides novel insights into the molecular mechanism of VA's antiaging and antioxidative effects and suggests that this micronutrient could be a potential therapeutic agent for age-related disorders.

1133B **A gain-of-function mutation in chondroitin polymerizing factor extends lifespan and healthspan** Yukimasa Shibata[1], Yuri Tanaka[1], Shion Fujii[1], Hiroyuki Hiroyuki Sassa[2], Hidenao Toyoda[3], Kiyoji Nishiwaki[11]Dept. of Biomed. Sci., Kwansei Gakuin Univ, [2]CDB, RIKEN, [3]Fac. Pharm. Sci., Ritsumeikan Univ

Aging impairs function of sensory, motor, digestive, and immune systems, and increases the risk of age-related diseases. Therefore, elucidating the mechanisms of aging and developing medical treatment to control progression of aging help to maintain healthy period by removing the cause of age-related diseases. It has become clear that aging causes damage of extracellular matrix (ECM), including crosslink, fragmentation, and change of composition, such as collagen and chondroitin. It is known that ECM not only serves as a scaffold for cells, but also regulates multiple biological processes. However, mechanism that regulates aging by ECM is not clear yet.

Interestingly, we found that integrity of ECM and endogenous chondroitin slows aging processes in the regulation of body length, defecation cycle, pumping, and lifespan. We examined mutants of the ADAMTS protease MIG-17, which is required for basement membrane reorganization, and found that aging progresses in *mig-17* mutants proceeded more rapidly than that in the wild type. The mutants of SQV-5, a chondroitin synthase, exhibited a shortened lifespan in addition to accelerated aging progression. We also found that gain-of-function mutation in the chondroitin polymerizing factor MIG-22 extended lifespan and healthspan of wild type animals. *mig-22gf*suppressed premature aging in *mig-17* mutants, suggesting that MIG-22 acts downstream of MIG-17. *mig-22gf* also suppressed the premature aging, but not the shortened lifespan of *sqv-5* mutants, suggesting that the lifespan extension in *mig-22gf* depends on *sqv-5*. Interestingly, although *mig-22gf* suppressed the premature aging of periodic behaviors in *sqv-5* mutants, it failed to suppress the chondroitin synthesis defect. These results indicate that the amount of chondroitin regulates lifespan and age-dependent reduction of body length, but not age-dependent decline of periodic behaviors.

1134B **Toxicological effects of 6PPD in *Caenorhabditis elegans*** moonjung hyun[1], Ho-jeong Lee[2], Kwang Hyun Hwang[2], Jeong-doo Heo[21]Gyeongnam Biohealth Research Center, Korea Institute of Toxicology, [2]Korea Institute of Toxicology

N-(1,3-dimethyl butyl)-N'-phenyl-p-phenylenediamine (6PPD) is a widely used rubber antioxidant with potentially lethal effects on wild and model fishes. However, its toxicity in other species and the underlying mechanism remain largely unknown. Here, we used the nematode Caenorhabditis elegans as an in vivo model system to investigate the impact of 6PPD on development, reproduction, health, and aging in vivo. Wild-type worms were exposed to 0, 0.5, and 1.0 mM 6PPD during development. Our results showed that 6PPD treatment increased embryo and larval lethality, reduced body length, decreased mobility, and slowed development. Moreover, 6PPD substantially decreased reproductive ability and shortened lifespan. Finally, we observed that 6PPD decreased mitochondria DNA copy number, oxygen consumption, and ATP level. These results suggest the potentially harmful physiological effects of 6PPD. Further studies will elucidate the potential mechanisms of toxicity of 6PPD at the molecular levels.

1135B **Comparison of toxicity of Bisphenol analogs in Caenorhabditis elegans** Moonjung Hyun, Ho-jeong Lee, Kwang Hyun Hwang, Jeong-doo Heo Korea Institute of Toxicology

Bisphenol A (BPA) is widely used in plastic food containers and baby products and accumulates in human bodies. BPA is classified as an endocrine-disrupting chemical and is associated with various diseases. Although many alternative BPA analogs have been developed, their potential toxicity has not been fully evaluated. In this study, we selected nine BPA substitutes, including bisphenol AF (BPAF), bisphenol B (BPB), bisphenol C (BPC), bisphenol TMC (BPTMC), bisphenol AP (BPAP), bisphenol C-dichloride (BPC2), bisphenol P (BPP), tetrabromobisphenol A (TBBPA), and bisphenol Z (BPZ), and analyzed their toxicity using the nematode Caenorhabditis elegans as an in vivo model system. Our findings indicate the potential toxicity effects of BPA alternatives. Further studies will elucidate the potential mechanisms of toxicity of these nine bisphenol substitutes at the molecular level.

1136B **Effect of DR and omega-3 EPA supplementation on Sarcopenia in *Caenorhabditis elegans*** Jana J Stastna[1], Marieke J Bloemink[1], Sobha Tumbapo[2], Simon C Harvey[21]Life Sciences, Canterbury Christ Church University UK, [2]Canterbury Christ Church University UK

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. Despite extensive research, sarcopenia is still not well understood at the molecular level, although multiple factors have been found to influence the development of sarcopenia, including physical inactivity, and an unbalanced diet.

Using *C. elegans* as a model system, this study aims to determine the effect of dietary interventions, like dietary restriction and dietary supplementation, on the rate of muscle loss. Our work uses the peptone withdrawal method and a transgenic *C. elegans* strain (unc-54::GFP), to quantify myosin density using fluorescence spectroscopy. We have developed a novel way to quantify the muscle loss by using an internal GFP standard for calibration. In addition, average lifespan, motility, and intestinal barrier function (IBF) were also determined at various dietary interventions.

Our data show that *C. elegans* exposed to mild and medium DR display an increase in lifespan and heath span, with a delayed onset of sarcopenia. Supplementation with sources of omega-3 EPA (eicosapentaenoic acid), has a similar effect, with improved lifespan and delayed sarcopenia. Age-related intestinal barrier function was also improved in EPA-supplemented worms, with

GC-MS confirming the uptake of omega-3 EPA. These results show that dietary intervention can delay the onset of age-related diseases such as sarcopenia and improve intestinal barrier function in *C. elegans*.

1137B **Programmed Cell Death Impaired in a *C. elegans* Short-lived Mutant Induces Recovery of Lifespan and Hypokinesia** Sumino Yanase[1], Michiyo Suzuki[2], Kayo Yasuda[3], Naoaki Ishii[41]School of Sports & Health Science, Daito Bunka University, [2]Takasaki Advanced Radiation Research Institute, National Institutes for Quantum Science and Technology, [3]Undergraduate School of Health Studie, Tokai University, [4]Undergraduate School of Health Studies, Tokai University

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 gene causes an increase in mitochondrial oxidative stress, thereby induces abnormal apoptosis in embryonic development and shortens the lifespan [1]. Here we examined the difference in age-related phenotypes between the short-lived *mev-1(kn1)* and *mev-1;ced-3* mutant, which has deficient programmed cell death. The *ced-3* mutation recovered the lifespan in *mev-1* mutant up to the lifespan in the wild-type [2]. Intriguingly, *mev-1;ced-3* mutant showed marked slow movement and recovery of hypersensitivity to oxidative stress. Here, we propose a novel role of programmed cell death signaling to the mitochondrial function in *C. elegans* during aging.

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1138B **Fighting parasitic nematodes with microbial crystals** Fathima S. Mohammed Refai[1], Hala Fahs[2], Rita Haber[3], Nancy Fayad[3], Clara Palazzolo[4], Zaynoun Attieh[5], Alan Twaddle[2], Suma Gopinadhan[5], Fabio Piano[6], Antony Page[7], Mireille Kallassy[3], Kristin C. Gunsalus[61]Science, New York University Abu Dhabi, [2]New York University, [3]Universite Saint-Joseph, [4]CGSB, New York University Abu Dhabi, [5]New York University Abu Dhabi, [6]New York University Abu Dhabi, New York University, [7]University of Glasgow

The discovery of new broad-spectrum anthelmintics to target parasitic worms, which affect 24% of humans, crops, and livestock, remains a challenge. We are using small molecules and natural products to identify novel compounds that affect nematodes and study their modes of action using the free-living nematode models *C. elegans* and the distantly related *P. pacificus*. *Bacillus thuringiensis* (Bt) is a spore-forming bacterium that synthesizes crystal inclusions, some of which are toxic against insects (bioinsecticides), nematodes and cancer cells. These toxins are safe to humans, biodegradable and constitute a promising alternative to chemical anthelmintics. We have screened a library of 300 uncharacterized strains of the Bt to identify crystal (Cry) proteins showing toxicity against nematodes. We found 95 strains that hinder the development of worms, among which 50 strains act through a mechanism independent of the known nematicidal toxin Cry5. Tests in the plant root-knot parasite *Meloidogyne* and the veterinary parasite *Haemonchus contortus* revealed 20 strains with variable severity effects. Virulence factors of these strains are being characterized by DNA sequencing combined with protein mass spectrometry and functional genomic assays to elucidate their mechanisms of action.

1139B **A versatile high-throughput chemical screening platform discovers a new natural anthelmintic compound** Yasmine Moussa[1], Hala Fahs[2], Fathima S. Refai[1], Suma Gopinadhan[3], Patricia G. Cipriani[4], Robert D. White[3], Stephan Kremb[3], Xin Xie[3], Yanthe Pearson[3], Antony Page[5], Fabio Piano[6], Kristin C. Gunsalus[41]Science, New York University Abu Dhabi, [2]Science, New York University, [3]CGSB, New York University Abu Dhabi, [4]CGSB, New York University, New York University Abu Dhabi, [5]University of Glasgow, [6]Science, New York University, New York University Abu Dhabi

Parasitic worms infect over 1.5 billion people and cause significant losses in livestock and crops. New anthelmintic drugs are needed, as resistance to existing drugs is emerging. We established a high-throughput and high-content robotic screening platform to identify bioactive small molecules and their molecular targets, focusing on novel disease therapeutics and broad spectrum anthelmintics. The high-throughput platform (HTP) can screen compound collections and RNAi libraries in model organisms and mammalian cells in a fully integrated manner. 35,000 compounds were screened for broad anthelmintic properties while being non-toxic to human cells. The screen identified most known anthelmintics and numerous new compounds, among which we discovered D1, a plant-derived compound that caused dose-dependent mortality in *C. elegans* and *P. pacificus* across different developmental stages, including embryos. This molecule also causes mortality in the multi-drug resistant parasite *Haemonchus contortus* UGA strain (ruminants). Phenotypic characterization in *C. elegans* revealed defects in embryonic elongation. D1-resistant strains generated by random mutagenesis with ethyl methanesulfonate (EMS) are susceptible to known anthelmintic drugs, indicating that the D1 compound acts through a different pathway. We are currently characterizing the mutations leading to resistance in these strains and will perform further molecular and genetic analyses to identify its mode of action.

1140B **Vitamin B12 protects *C. elegans* from thiol toxicity**

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We have identified a novel SAM methyl transferase, RIPS-1, that is upregulated in the gut and hypodermis by thiol reducing agents and hydrogen sulfide, with expression controlled by the hypoxia inducible factor pathway. Homologues of RIPS-1 are found in a small subset of eukaryotes and bacteria, many of which can adapt to fluctuations in environmental oxygen levels. We identified *rips-1* through forward genetic screens as the sole gene that when mutated allowed worms to survive normally lethal concentrations of thiol reducing agents such as dithiothreitol (DTT) and b-mercaptoethanol. DTT toxicity can be overcome by loss of RIPS-1, by using growth conditions that provide higher levels of vitamin B12 and by methionine supplementation. A targeted RNAi modifier screen identified the mitochondrial enzyme methylmalonyl-CoA epimerase as a strong genetic enhancer of RIPS-1 mutant resistance to DTT. This work highlights the central importance of dietary vitamin B12 in normal metabolic processes in *C. elegans*, defines a new role for this vitamin in countering reductive stress and identifies RIPS-1 as a novel HIF-1 induced methyl transferase in the methionine cycle.

1141B **A drug development platform from bench to bedside for the treatment of age-related diseases** Matthias Rieckher[1], Caterina Tezze[2], Ann TJ Belien[1], Marco Sandri[2], Evi M Mercken[11]Rejuvenate Biomed, [2]Department of Biomedical Sciences, University of Padova

Diseases of aging pose a major challenge to health care systems due to an increasing elderly population, urgently requiring the development of effective drugs targeting age-related diseases. Working with compounds that have human safety at hand, Rejuvenate Biomed developed a unique proprietary *in silico* platform that is employed to identify novel drug combinations, which are predicted to synergistically impact molecular pathways involved in age-related diseases. An in-house *C. elegans* platform is used to verify the prediction in aging and in disease models, before moving the drug combinations in genetic mouse models and old mice for further substantiation. Subsequently, a mechanistic clinical study provides insights on the efficacy in humans. Based on this approach, a first drug combination was identified targeting sarcopenia. Sarcopenia is characterized by a progressive loss of physical performance and skeletal muscle mass and strength, causing frailty and disability, with no effective treatment available thus far. Applying this approach, the first *in silico* predicted drug combination named RJx-01 (combining compounds A and B) was tested in *C. elegans*, demonstrating improved lifespan, locomotion, pharyngeal pumping, and muscle fiber organization. Drug synergism was then validated in transgenic mice carrying a deletion in the mitochondrial protein optic atrophy 1 (Opa1), which results in severe age-related muscle loss. RJx-01 ameliorated physical performance, muscle mass and strength, as well as improved neuromuscular function in this model. Similarly, RJx-01 improved physical performance and muscle strength in 22-month-old wild type mice. The results support a beneficial synergistic effect of RJx-01. Currently, a disuse-induced muscle atrophy proof-of-mechanism Phase Ib study is ongoing within which RJx-01 is investigated. This clinical trial is designed to (1) evaluate the safety and tolerability of RJx-01 in older male subjects; and (2) determine pharmacokinetics of RJx-01 and (3) assess the pharmacodynamics effects of RJx-01.

1142B **Very long chain alkyl ascarosides as biosynthetic intermediates in ascaroside biosynthesis of *C. elegans*** Rocio Rivera Sanchez, Hanna Laaroussi, Stephan H. von Reuss Institute of Chemistry, University of Neuchâtel

Development and behavior of nematodes, such as the model organism *C. elegans*, are modulated by ascarosides, glycolipids of the 3,6-dideoxysugar L-ascarylose linked to (ω–1)-hydroxy fatty acid type aglycones. Ascaroside biogenesis depends on the co-option of the peroxisomal fatty acid β-oxidation cycle, which shortens the aglycones and thereby creates homologous series of acyl ascarosides with diverse biological functions. Comparative analysis of β-oxidation mutants revealed very long chain ascarosides (VLCAs) with up to 33 carbons as precursors for the short chain signaling molecules, but the biosynthetic origin of these VLCAs has remained enigmatic [1].

We have previously employed mixed isotope labelling experiments, which indicated that the VLCAs are derived from glycosylation and decarboxylation of very long chain (*S*)-3-hydroxy fatty acids (and not from (ω–1)-hydroxy fatty acids), thus, suggesting the implication of very long chain alkyl ascarosides as potential biosynthetic intermediates [2].

Here, we describe the identification of these predicted very long chain alkyl ascarosides (called asc-C#-H) in the lipidome of *C. elegans* (and other nematodes) by comparative GC-MS and HPLC-MS analysis with synthetic standards. We show that the *de novo* biosynthesized branched-chain *iso*-acyl and *iso*-alkyl ascarosides, such as asc-*i*C26 and asc-*i*C26-H, are both [$^{13}C_5$]-labelled upon incorporation of a L-[U-$^{13}C_6$]-leucine enriched *E. coli Δile Δleu Δval* mutant, and demonstrate that the *elo-5(gk208)* loss of function mutant utilizes the branched chain fatty acid *iso*-C17 as an intermediate in asc-*i*C26-H biosynthesis, although some alternative *elo-5* independent pathway is dominating. Finally, comparative metabolomics with diverse *C. elegans* mutants previously implicated in lipogenesis or fatty acid metabolism revealed that the dominating alkyl ascarosides asc-C25-H and asc-*i*C26-H are

enriched in *cyp-37A(ssd9)* and *emb-8*(*ssd89*) (previously reported to produce supersized lipid droplets [3]), suggesting that cytochrome *cyp-37A (drop-1)* and the cytochrome P450 reductase *emb-8* (*drop-8*) are involved in the oxidation of alkyl ascarosides and, thus, represent one of several entry points into short chain ascaroside biogenesis upstream of the peroxisomal β-oxidation cycle. Considering that alkyl ascarosides have long been considered to be implicated in the resistance of nematode eggs versus environmental factors, our results provide a first link between these germline-related physiological functions and their established role as small molecule signals in nematode chemical ecology.

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1143B **The 1 –Cys peroxiredoxin, PRDX-6, suppresses a pro-survival response, including the Flavin monoxygenase, FMO-2, that protects against bacterial and fungal infection** Elizabeth L Veal, Emma L Button, Jake L Lewis, Elise Bennett, Emilia Dwyer
Biosciences Institute, Newcastle University

Reactive oxygen species (ROS)-induced cell damage can lead to a range of diseases, however controlled production of ROS is important for cell signalling and innate immunity. Hence, there is great interest in understanding how the damaging and positive functions of ROS are regulated. Peroxiredoxins (Prdx) are ubiquitous peroxidases that utilise reversibly oxidised cysteines in the catalytic detoxification of peroxides but play other important roles in ROS responses. Here we show that, unexpectedly, *C. elegans* lacking the 1-Cys Prdx *prdx-6,* have an increased resistance to oxidative stress and extended lifespan under some conditions. Moreover, *prdx-6* mutant worms are also more resistant to infection with two opportunistic human pathogens; the gram positive bacteria *Staphylococcus aureus* and the dimorphic yeast *Candida albicans*. Our data suggest that increased levels of ROS in *prdx-6* mutant worms lead to increased germline apoptosis and expression of the Flavin monoxygenase, FMO-2. Indeed, we show that FMO-2 is induced as part of an NHR-49-mediated protective response to *C. albicans* and *S. aureus*. Accordingly, our data suggest that elevated *fmo-2* expression, protects *C. elegans* against both of these opportunistic human pathogens, and may be important for the increased lifespan and innate immunity of *prdx-6* mutant animals. This reveals a new role for this Flavin monoxgenase in protecting against fungal infection and adds to increasing evidence that ROS and Prdx play complex roles in regulating stress resistance, immunity and ageing.

1144B **From monomers to amyloids – Insights from a novel C.elegans tau model** Franziska Hirsch[1], Janine Kirstein[1,2]1Faculty 2, Cell biology, University of Bremen, [2]Leibniz Institute of Aging - Fritz Lipmann Institute, Jena, Germany

Alzheimer's disease is a progressive neurodegenerative disease that manifests by two main pathological hallmarks, which are extracellular plaques of the Amyloid-beta peptide and neurofibrillary tangles of the tau protein largely found intracellularly that spread in a prion-like mechanism from one cell to another. However, our knowledge about molecular processes and mechanisms leading to the disease are not fully understood, in particular debating whether amyloid propagation is the cause or the consequence of the pathogenic onset.

To overcome this obstacle, we have recently developed a novel *C. elegans* disease model mimicking AD pathology by expressing the human tau protein in the nervous system of *C. elegans* under control of the pan-neuronal promotor *rgef-1* as well as a second tau moiety C-terminally fused to mScarlet, which is expressed sub-stoichiometrically driven by the same promoter, but under the control of an internal ribosome entry site (IRES). Using this labelling strategy we generated two distinct strains, one expressing the wild-type human tau whereas for the other strains a specific tau variant was used harbouring the two frontotemporal dementia-associated mutations P301L and V337M. These novel models allow to track the expression, aggregation and spreading of the disease protein *in vivo* in a near native manner.

Employing fluorescence lifetime imaging microscopy enabled us to quantify tau amyloid fibrils with the progression of ageing in both strains. We observed a significant increase of aggregates from Day 4 to Day 10 of the nematodes' life of the neuronal Tau mutant strain compared to the neuronal Tau wild-type and nScarlet control strain, which both displayed no significant aggregation among ageing. We could further observe that tau is able to spread to distal tissues and was found to be transported to the coelomocytes. Notably, the abundance of tau increases as the animal ages, which is on contrast to other neurodegenerative disease models of *C. elegans*.

Based on these findings our established model is very well suited to study physiological defects, neuronal dysfunction and neurodegeneration that occur upon tau aggregation and spreading and allows to investigate the consequences of traumatic brain injury, which are hypothesized to produce tau seeds enhancing pathogenic spreading and aggregation

1145B **Non-Autonomous Regulation of Mitochondrial Connectivity through Tyramine Signaling, HLH-30/TFEB and Autophagy** Yu-Cheng Chang, En-Ni Chang, Chun-Liang PanInstitute of Molecular Medicine and Center for Precision Medicine, College of Medicine, National Taiwan University

Non-Autonomous Regulation of Mitochondrial Connectivity through Tyramine Signaling, HLH-30/TFEB and Autophagy Intercellular regulation of mitochondrial morphology and function is important for physiological homeostasis at the organismal level. Our previous study found that inhibition of mitochondrial fusion in C. elegans neurons, via depletion of fzo-1/Mitofusin, triggers systemic mitochondrial fragmentation in distal non-neural tissues through serotonin and tyramine. This non-autonomous control of mitochondrial connectivity engages autophagy/mitophagy genes and enhances pathogen immunity. Here we show that tyramine promotes non-autonomous mitochondrial fragmentation in the intestine. It does so by targeting the TYRA-3 G-protein coupled receptor in intestinal cells, or via LGC-55, a tyramine-gated chloride channel, in the neurons. Tyramine-TYRA-3 signaling likely engages EGL-30, the sole C. elegans G alpha q. Both metabotropic and ionotropic tyraminergic signaling promotes nuclear translocation of HLH-30/TFEB, a transcription factor required for lysosome and autophagy function, in the intestine. We previously found that autophagy genes are upregulated by neuronal fzo-1 depletion. Extending this observation, we showed here that sqst-1/p62, atg-9, epg-9 and dct-1/BNIP3 are required for non-autonomous mitochondrial fragmentation triggered by neuronal fzo-1 depletion. Our findings uncover a tyramine-TFEB-autophagy signaling axis for brain-gut communication of mitochondrial connectivity regulation.

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1146B **Identify the function of male seminal fluid protein SFP-1 on the regulation of hermaphrodite longevity in *Caenorhabditis elegans*** mingqing Chen huazhong university of science and technology

Sexual intercourse negatively influence health and longevity in many species, while seminal fluid has been shown to play a significant role in this process. *Caenorhabditis. elegans* has been established as a good model to study how intense sexual intercourse affect longevity. However, the mechanism of the seminal fluid induced post-mating death and fat loss is still unclear. In this study, we focused on the function of the protein SFP-1 which specifically expresses in the seminal vesical of males and is secreted into seminal fluid. We revealed the hermaphrodite mated with *sfp-1* mutant males had longer lifespan than mated with wild-type, which indicated the secreted protein was involved in post-mating death. Then, we expressed the protein in the hermaphrodite's intestine, muscle, germline, and nervous cells respectively, and found out that only intestine over expression (OE) can shorten the transgene line lifespan. This result indicated that the SFP-1 is important for the regulation of mated hermaphrodite lifespan through the intestine cells. Furthermore, to clarify the key domain of SFP-1, we constructed a variety of truncations expressed in the intestine and measured the lifespan of these transgene lines, suggested NTF2-like domain as a crucial element for SFP-1 function. To identify transcription factor in regulating aging in intestinal OE., we used RNAi screen and found SKN-1 RNAi can significantly extend the lifespan. Moreover, RNA-seq revealed that *gst-38* was up-regulated by intestinal OE. Together, these results suggest that SFP-1 activates SKN-1, which promotes the nuclear localization in intestinal cell, then significantly increased the expression level of GST-38. The mammalian homolog of GST-38, hematopoietic prostaglandin D synthase, is known for synthesis prostaglandin (PG). In *C. elegans*, F-series prostaglandin functions in sperm guiding and is produced from polyunsaturated fatty acid (PUFA) precursors. Here, we found the *sfp-1* mutant males have defects in sperm guiding and exogenous PG rescues this phenotype. Meanwhile, sexual intercourse induces alters in lipid metabolism and promote fat loss in hermaphrodite. We found that intestinal OE induced fat loss severely, and the lipid metabolism genes *fat-2* and *fat-6* involved in longevity mediated by SFP-1. These results indicated that SFP-1 initiates the consumption of intestinal lipid to synthesize PG, thereby mediating male sperm guiding. In summary, the male specific secreted protein SFP-1 is essential for post-mating death. It is transferred from the seminal fluid and targeted to the intestine to regulate the hermaphrodite longevity, PG synthesis and sperm guiding.

1147B **Elucidating a mitochondria-to-nucleus signaling pathway that promotes longevity** ULRICH ANGLAS, Jeremy Van RaamsdonkIntegrated Program in Neuroscience, McGill

Understanding aging could aid in delaying both age-associated diseases and the overall decreased quality of life associated with aging. One prominent aging theory, the free radical theory of aging, proposes that organisms age due to the build-up of oxidative damage caused by reactive oxygen species (ROS). However, we have shown that disruption of the mitochondrial antioxidant protein, superoxide dismutase 2 (SOD-2), increases lifespan in *C. elegans*. To determine whether this increased lifespan is due to increased mitochondrial ROS, we examined the effect of increasing superoxide levels through treatment with paraquat or decreasing superoxide levels through treatment with antioxidants. We found that treating wild-type worms with paraquat is sufficient to extend longevity. In contrast, all concentrations of paraquat decreased lifespan in *sod-2* mutants suggesting that *sod-2* animals may already have the optimal level of ROS for enhanced longevity. This conclusion is supported by our observation that treatment with antioxidants decreases *sod-2* lifespan while having minimal impact on the lifespan of wild-type worms. Our

current project is to elucidate the molecular mechanism through which mild elevation of mitochondrial ROS increases lifespan. We hypothesize that increased ROS in the mitochondria acts as a stress signal that is communicated to the nucleus to protect the organism from ROS stress and that the resulting changes in gene expression cause extended longevity. We further hypothesize that intracellular signals between the mitochondria and nucleus are transmitted through kinase signalling. To test this, I disrupted the individual expression of 91 kinases in *sod-2* worms to identify kinases that are required for *sod-2* longevity. I identified a number of kinases that are required for the long lifespan of *sod-2* mutants, supporting our model. The next step is to determine whether these kinases influence longevity in other long-lived mutants and if they are also involved in stress pathways. I will also use RNA sequencing to identify changes in gene expression present in *sod-2* mutants that are prevented by inhibiting signaling from kinases required for *sod-2* longevity. This work will advance our understanding of the mechanisms involved in the aging process.

1148B **Intracellular calcium mediates neuronal mitophagy upon stress** Antonis Roussos[1], Konstantinos Palikaras[21]Department of Physiology, Medical School, National and Kapodistrian University of Athens, [2]Department of Physiology Medical School, National and Kapodistrian University of Athens

Mitochondria are essential for energy production and have vital roles in calcium signaling and storage, metabolite synthesis and apoptosis, among others, in eukaryotic cells. Neuronal cells are particularly dependent on proper mitochondrial function. Thus, maintenance of neuronal homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the elimination of damaged or superfluous mitochondria. Mitochondrial impairment has been implicated in several age-related neurodegenerative diseases. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the effects of mitophagy deficiency in neuronal physiology. To address this question, we developed two composite, in vivo imaging approaches to monitor mitophagy in neurons. Neuronal mitophagy is induced in response to challenged conditions. Mitochondrial dysfunction leads to transportation of axonal mitochondria towards the neuronal cell body, in calcium- and an AMPK-dependent manner. Autophagy deficiency increases mitochondrial number in neurons of age-matched nematodes and abolishes mitochondrial axonal transport upon stress. Additionally, impairment of mitophagy results in enhanced cell death in C. elegans models of neurodegeneration. Our results indicate that mitophagy contributes to preserve mitochondrial homeostasis and neuronal health.

1149B **Inactivation of HSF-1 results in the age dependent increase of thermotolerance mediated by the unfolded protein response of the ER** Dániel Kovács[1], Barnabás J Bíró[1], Tímea Sigmond[1], Bernadette Hotzi[1], Saqib Ahmed[1], Mohammad Umar[1], Márton Kovács[1], Tibor Vellai[1,2], János Barna[1,21]Department of Genetics, Institute of Biology, Eötvös Loránd University, [2]MTA-ELTE Genetics Research Group, Eötvös Loránd University

In living organisms, several protective mechanisms have evolved to alleviate the proteotoxic effects of distinct environmental stresses. One of these mechanisms is called the heat shock response. The master regulator of the heat shock response in *Caenorhabditis elegans* is the heat shock transcription factor CeHSF-1, a conserved transcription factor in eukaryotes. HSF1 helps to refold damaged proteins by the activation of chaperone genes. Furthermore, it has a protective role in the progress of aging and neurodegenerative diseases via the maintenance the cellular proteostasis. HSF1 has also been implicated in carcinogenesis and tumor progression. Manipulating HSF1 activity bears a promising therapeutic potential for these disorders. Thus, it is crucial to determine the overall impact of altered HSF1 activity on an organism. Lack of CeHSF-1 activity is usually associated with impaired stress tolerance, however here we show that inactivation of CeHSF-1 results in increased thermotolerance in young adult worms. As worms grow older CeHSF-1 depleted animals become less resistant to heat than the wild type suggesting that the impact of HSF1 depletion on thermotolerance is age dependent. To find out what mechanism could cause such an unexpected result we performed an RNAseq analysis and several expression analyses of stress induced reporters. Our data supports that in young adult worms distinct cellular stress pathways including the insulin-like signaling pathway and the unfolded protein response is induced upon CeHSF-1 deficiency. We also tested several strains with impaired stress pathways to see whether the increased thermotolerance of CeHSF-1 depleted young adult worms is suppressed under these conditions. The unfolded protein response can be activated by three redundant pathways. We found that simply inhibiting one pathway did not suppress the increased thermotolerance phenotype of the *hsf-1* mutant worms. This is most likely due to the redundant nature of these three pathways. Inhibiting two of the pathways in a *hsf-1* mutant background resulted in a significant decrease in thermotolerance. This leads us to believe that the unfolded protein response is the cellular stress response pathway responsible for compensating for the loss of the heat shock response.
It is plausible that similar compensatory mechanisms also operate in higher organisms raising the possibility of an unexpected outcome when activity of HSF1 is manipulated.

Keywords: autophagy, *C. elegans*, cellular stress response, heat shock factor 1, heat shock proteins, heat shock response, insulin-like signaling pathway, unfolded protein response

1150B ***kri-1* and *ccm-3* Cooperate in Regulation of Innate Immunity** Samuel J Krempel[1,2], Michelle Glossop[2], Bin Yu[1], Brent Derry[1,21]Developmental and Stem Cell Biology, Hospital for Sick Children, [2]Department of Molecular Genetics, University of Toronto

Cerebral Cavernous Malformations (CCM) is a neurovascular disease caused by loss of function mutations in KRIT1/CCM1, CCM2 and CCM3 which leads to raspberry-like malformations in the blood vessels of the central nervous system. In *C. elegans,* the KRIT1 ortholog *kri-1* and the CCM2 ortholog *ccm-2* regulate the *mekk-3/mek-5/mpk-2* kinase cascade and the transcription factor *klf-3* to regulate starvation survival and germline irradiation induced apoptosis. The CCM3 ortholog *ccm-3* acts within the striatin-interacting phosphatase and kinase (STRIPAK) complex to maintain integrity of biological tubes, including the excretory canal and the the germline. The 3 CCM proteins appear to regulate two distinct signaling pathways that share no known common phenotypes or downstream effectors, however because loss of *kri-1* is synthetic lethal with loss of *ccm-3* I hypothesized they may converge to co-regulate some as of yet unknown essential pathway or function. Using the Auxin-Inducible Degradation (AID) system to conditionally ablate *ccm-3* in *kri-1* mutants, I found that ablation of *kri-1* and *ccm-3* caused severe intestinal defects and intestinal leakage. This synthetic lethality was rescued by raising worms on lipopolysaccharide (LPS) free *E. coli* food, as well as deletion of two components of the mediator complex previously shown to regulate innate immunity. Knockdown of the canonical LPS response pathway also phenocopied loss of *ccm-3.* In light of recent studies that implicate innate immunity defects in the pathogenesis of CCM disease where barrier integrity is compromised in the brain microvasculature of CCM patients, these results suggest *kri-1* and *ccm-3* converge to regulate innate immunity in the worm, which may be required for intestinal barrier function and viability in the worm. With these findings, *C. elegans* provides a useful model for delineating the cellular functions of CCM genes and the role of innate immunity in CCM disease.

1151B **Increasing Mitochondrial Fission and Fusion Capacity Enhances Biological Resilience and Extends Lifespan** Annika Traa[1,2,3], Zenith Rudich[1,2,3], Jeremy Van Raamsdonk[1,2,3,41]Neurology and Neurosurgery, McGill University, [2]Metabolic Disorders and Complications Program, Research Institute of the McGill University Health Centre, [3]Brain Repair and Integrative Neuroscience Program, Research Institute of the McGill University Health Centre, [4]4Division of Experimental Medicine, McGill University

Mitochondria interact with each other to form a dynamic network that undergoes fission and fusion in order to change its conformation depending on the metabolic and energy needs of the cell. Mitochondrial fission is required for mitophagy, distribution of mitochondria to subcellular locations and proliferation of mitochondria during cell division. Mitochondrial fusion allows for complementation of mitochondrial components in order to maintain mitochondrial function. Previously, we have reported that in *C. elegans*, mitochondrial networks are tubular, interconnected and organized in young and healthy worms, but become fragmented and disorganized with age and in models of age-associated neurodegenerative disease. In this work, we determine whether increasing mitochondrial fission or mitochondrial fusion capacity can maintain mitochondrial network homeostasis during aging, improve resilience and extend lifespan. To increase mitochondrial fission and fusion capacity, we ubiquitously overexpressed the mitochondrial fission gene *drp-1* or the mitochondrial fusion genes *fzo-1* and *eat-3*. We then measured physiologic rates, lifespan, stress resistance, mitochondrial function, ROS production and mitochondrial network morphology in order to characterize the effects of overexpression of either fission machinery, fusion machinery or both fission and fusion machinery in *C. elegans*. Surprisingly, we found that overexpression of either fission or fusion machinery extended lifespan and increased stress resistance despite causing an increase in mitochondrial fragmentation. Furthermore, overexpression of the mitochondrial fission gene *drp-1* at the same time as either of the mitochondrial fusion genes does not revert the phenotype of the mitochondrial fusion overexpression strains to wild-type. Overall, our results demonstrate that increasing mitochondrial fission of fusion capacity can extend lifespan and improve biological resilience without promoting the maintenance of a youthful mitochondrial network morphology.

1152B **Chemical solvent dihydrolevoglucosenone extends lifespan and ameliorates deficits in neurodegenerative disease models** Abdelrahman AlOkda[1,2,3], Jeremy M. Van Raamsdonk[1,2,3,41]Neurology and Neurosurgery, McGill University, [2]Metabolic Disorders and Complications Program, Research Institute of the McGill University Health Centre, [3]Brain Repair and Integrative Neuroscience Program, Research Institute of the McGill University Health Centre, [4]Division of Experimental Medicine, McGill University

Aging is the main risk factor for developing age-related diseases such cardiovascular disease, cancer, and neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's disease. Given the increase of age-related diseases in the general population, it is crucial that we advance our understanding of the process of aging to promote healthy aging, and to reduce age-related diseases. In order to identify neuroprotective compounds, I first examined the effect of different solvents on *C. elegans* physiology. Surprisingly, I found that administration of dihydrolevoglucosenone (known as Cyrene), a new environmentally safe and biobased solvent, increases mean and maximum lifespan by 65% and 48%, respectively. Cyrene treatment was also able to reverse the age-associated decline in mobility in wild-type worms. As our work and others have shown a strong association

between stress resistance and lifespan, I also examined the effect of Cyrene on stress resistance and found that it increases resistance to several stressors including oxidative, osmotic and UV stress. Since previous studies by our lab and others has shown that genes and interventions that increase lifespan can be neuroprotective, I examined the effect of Cyrene in *C. elegans* models of neurodegenerative disease and found that Cyrene significantly increases mobility in animal models of Alzheimer's, Parkinson's, and Huntington's disease. My ongoing research aims to identify the molecular mechanisms by which Cyrene prolongs life and improves health and determine the impact of manipulating these mechanisms on lifespan and healthspan. This work will provide novel insight into biological pathways influencing aging, and how we can modulate them as a potential therapeutic strategy to promote healthy aging and limit age-related diseases.

1153B **Using *Pristionchus* nematodes to better understand the molecular and functional effects of plant-derived dietary antioxidants on health and ageing** Rebekah J White, Cameron Weadick Biosciences, University of Exeter

In humans, tart cherry supplement improves muscle recovery due to upregulated antioxidant gene expression (Wangdi et al. 2021). Following this supplement, the antioxidants showing highest increase in blood are hippuric acid (HA), vanillic acid (VA), and 4-hydroxybenzoic acid (4-H). It is unclear which, if any, of these antioxidants result in this improved recovery. Here, to explore the functional and molecular effects of HA, VA, and 4-H, *Pristionchus* nematodes are used as model systems. Kaplan-Meier survival assays were run to record effects on worm ageing and longevity, and rupturing frequency is used as a quantitative indicator of healthspan (Leiser et al. 2016).

Evidence for effects on *Pristionchus* health was mixed. Longevity was unchanged, but with HA, reduced rupturing was observed in *Pristionchus pacificus*, indicating improved healthspan. Additionally, increased fertility was recorded – while rupturing may be due to age-linked muscle weakness or associated with egg-laying, we show that HA-supplemented *P. pacificus* produce more offspring than untreated worms. Rupturing may also be associated with age-linked cuticle thickening, perhaps due to increased muscle force required to lay eggs through the narrowed vulva opening. Using transmission electron microscopy, we compare the cuticle ultrastructure of HA-treated and untreated worms to discern if this plays a role in decreased rupture frequency. To gain insight into gene function and systems biology associated with these changes, we use RNA-seq to uncover differentially expressed genes and discuss how applicable this may be to other organisms.

In a wider context, understanding the effects of HA in *P. pacificus* at the molecular and functional level will help direct drug development work with this compound, and provide insight into the genetic basis of age-linked decline.

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Wangdi JT, O'Leary MF, Kelly VG, Jackman SR, Tang JCY, Dutton J, Bowtell JL (2021) Tart Cherry Supplement Enhances Skeletal Muscle Glutathione Peroxidase Expression and Functional Recovery after Muscle Damage. *Medicine & Science in Sports & Exercise* 54: 609–621.

1154B **Elucidating actin dynamics in aging and its role in Alzheimer's disease pathology** Sudarson Baskaran[1], Sanjana Junnarkar[2], Christian Gallrein[3], Janine Kirstein[11]Leibniz Institute on Aging – Fritz Lipmann Institute, [2]Cell Biology, University of Bremen, [3]Leibniz Institute of Molecular Pharmacology, Berlin, Germany

Alzheimer's disease (AD) is the predominant cause of dementia worldwide. It is characterized by the accumulation of amyloid and tau proteins leading to synaptic dysfunction in neurons. Dendritic spines communicate the majority of excitatory signals from postsynaptic neurons and the health of the dendritic spines is highly correlated with memory and neuronal function. The reduction in dendritic spine number and morphology has been associated with AD. The structure of these spines is mainly maintained by a robust network of actin filaments and their associated proteins. These modulators regulate actin dynamics and protect the dendritic spines from AD-associated oxidative and proteotoxic stress. Thus, the study of interplay between actin, actin-binding proteins and their effects on the structure and function dendritic spines enables us to better understand their role synaptic plasticity in AD. Previous studies revealed a protective role of the actin binding protein Drebrin (DBN) in dendritic spines. DBN-Ser647-P protects the filamentous actin (F-actin) from reactive oxygen species (ROS) induced oxidative stress (Kreis & Gallrein et al., 2019 Nat. Comm.). In order to study how DBN exerts its protective role on actin dynamics in AD pathology, we use genetically encoded tools that promote and perturb F-actin formation. DeActs (Disassembly-promoting encodable Actin tools) are genetic tools used for inhibition of F-actin. These DeActs molecules (e.g.GS1, SpvB) will be expressed in neurons of *C. elegans* under the control of an inducible system such as a splice switch that enables a temporal control over F-actin modulation. To complement this approach, we employ tools to stabilize of F-actin such as LifeAct that binds and stabilizes F-actin. These tools are currently being established and will be used to modulate actin dynamics to assess its contribution to Aβ aggregation, spreading and proteotoxicity. Decline in neuronal activity is correlated with oxidative stress and aging is characterized by a perturbed redox homeostasis. Yet, little is known how ROS affects neuronal activity and how activated DBN can preserve neuronal function with

the progression of aging. **This work aims to understand the specific mechanisms, regulations and upstream factors that play a role in Drebrin mediated protection of the actin network against oxidative and proteotoxic stress in AD pathology.**

1155B **Validation and optimization of an automated stress resistance screening method: LFASS** Sven Bulterijs, Bart P Braeckman Biology, Ghent University

Many, but not all, interventions that increase lifespan in *Caenorhabditis elegans* concomitantly increase resistance to various stressors. Therefore, stress resistance has been employed as a surrogate biomarker to screen for mutants with extended lifespan. However, these manual stress resistance assays are very laborious, suffer from low throughput as well as low temporal resolution. Furthermore, they are susceptible to experimenter bias and because death is determined by the cessation of movement, paralyzed worms could wrongly be classified as dead. Recently, a new method (named LFASS for Label-Free Automated Survival Scoring) was developed (Benedetto *et al.*, 2019) that allows for monitoring of population survival under acute stress in 96-well or even 384-well plates. This method exploits the short burst of blue 'death fluorescence' that occurs at the moment of death to determine the median time of death. The goal of our study was to optimize and validate this assay as a screening tool. We demonstrate that care has to be taken to avoid plate effects, but that high replicability is achievable if sufficient technical replicates are being used. We also demonstrate that this method is not compatible with certain stressors such as osmotic stress and dithiothreitol (DTT)-induced proteotoxic stress. Finally, we demonstrate a complex interaction between well volume, the stressor used and survival.

Benedetto A, Bambade T, Au C, Tullet JMA, Monkhouse J, Dang H, Cetnar K, Chan B, Cabreiro F, Gems D (2019). New label-free automated survival assays reveal unexpected stress resistance patterns during *C. elegans* aging. Aging Cell 18(5): e12998.

1156B **Neuronal BLI-4 modulates axenic dietary restriction mediated longevity in *C.elegans*.** Ping wu, Lieselot Vandemeulebroucke, Huai han Cai Gent university

Neuronal BLI-4 modulates axenic dietary restriction mediated longevity in *C.elegans*.

Ping Wu, Lieselot Vandemeulebroucke, Huaihan Cai, Bart P. Braeckman.

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When cultured in axenic medium, *C. elegans* display a dietary restriction-like phenotype that includes slow development, a slender appearance, and reduced fecundity. Hence, the term axenic dietary restriction (ADR) is often applied. ADR is a DR regimen that robustly extends lifespan of *C.elegans,* but the underlying molecular mechanism responsible for this longevity phenotype is still understudied.

Our previous transcriptomics analysis showed that many neuropeptide genes are significantly upregulated in axenically cultured worms. In addition, earlier results showed that neuronal CBP-1 is required for axenic longevity, hinting at a possible involvement of neuronal signaling in ADR-induced longevity. To verify this, we conducted a preliminary genetic screen of genes involved in the neuroendocrine signaling pathway. We found that RNAi knockdown of only the proprotein convertase *bli-4* can completely abolish ADR-induced longevity. Moreover, tissue specific knockdown approaches reveal that BLI-4 also acts in neurons to support ADR longevity.

As some neuropeptides, cleaved by BLI-4, are involved in the perception of food in certain neuronal circuits, we hypotheses that BLI-4 can may be involved in the processing of food sensory cues to mediate ADR longevity. Against our expectation, BLI-4 inactivation does not affect the animal's food sensing capability as tested with a food taxis assay. Additionally, lifespan rescue upon *bli-4* knockdown only occurs in standard axenic medium, but not in dilutions of axenic medium or other DR regimes such as bacterial dilution that also extend lifespan. These results suggest that the action of *bli-4* is very specific to diet type but is not necessarily involved in nutrient or bacterial cue sensing to increase ADR longevity.

1157B **What is the biological basis of Gompertzian aging in *C. elegans*?** Bruce Zhang, David GemsGenetics, Evolution and Environment, UCL

The application of demographic methods to understand the biology of aging has rather fallen from fashion since a peak of interest in the early 2000s. This may reflect the lack of success using this approach to achieve those stated aims. For example, the scale and rate parameters of the Gompertz model of age-related mortality rate are often interpreted as a population's baseline hazard and ageing rate, respectively. Yet such biological interpretations of demographic data are difficult to verify, particularly given the distinction between population-level (demographic) and individual-level (biological) phenomena.

Using *C. elegans*, we are attempting to better understand the biological basis of demographic parameters, by manipulating

factors that affect lifespan in, in particular, known causes of late-life mortality (including late-life diseases). This involves pairing demographic methodology with pathology analysis, which includes mortality deconvolution - the combined analysis of mortality and necropsy (i.e. pathology) data (1). Interventions used include alterations in insulin/IGF-1 signaling, pathogenicity of the *E. coli* food source, and culture temperature, alone or in combination. Interim results will be presented.

(1) Zhao, Y. et al. Two forms of death in aging *Caenorhabditis elegans*. *Nat. Commun*. 8, 15458 (2017).

1158B **Identification of virulence factors required for cell-cell spreading of the intracellular bacteria *Bordetella atropi* in the nematode *Oscheius tipulae*** Tuan D Tran, Makaela Levine, Robert Luallen Biology, San Diego State University

Bordetella atropi is an intracellular bacterial pathogen infecting the intestine of the host nematode *Oscheius tipulae*. We have shown previously that upon invading the host intestinal cells, *B. atropi* forms long filaments that pass through multiple intestinal cells for intracellular spreading. During this process, the host cortical actin appears to be disrupted as *B. atropi* filaments crossing the cell-cell boundary. We hypothesized that the force of the growing filaments alone may not be sufficient for breaking the cell-cell cortical actin and membrane, and therefore additional factors may be required to mediate filament crossing. To investigate this possibility, we first took a in silico approach to scan *B. atropi* genome for putative virulence factors and molecular machineries that could deliver effectors. We found that *B. atropi* encodes for one type III secretion system (T3SS) and two type VI secretion systems (T6SSs), which are molecular structures that can directly deliver effectors into host cells and have been implicated in pathogenesis of many clinically relevant bacteria, such as *Salmonella*Typhimurium, *Burkholderia spp.*, *Rickettsia spp.*, and *Pseudomonas aeruginosa*. In *B. atropi*, we knocked out key components of the two T6SSs and found no infection phenotype in *O. tipulae*, as the T6SS KOs had similar infection progression compared to the wildtype. By contrast, a *B. atopi* knockout mutant of the T3SS lost its capacity to invade host cells for intracellular infection, suggesting that *B. atropi* possesses a functional T3SS that is required for host cell invasion. Co-infection of T3SS mutant and wild-type strains did not result in invasion of T3SS-knockout bacteria, suggesting that effector(s) required for invasion do not have an endocrine-like effect, in agreement with the notion that activation of T3SS requires close contact between bacteria and host cells. We plan to directly inject the T3SS mutant into host intestinal cells to further examine the potential role of the T3SS downstream of host cell invasion.

Separately, we have found that *B. atropi* also harbors several putative effectors that potentially function in the capacity for bacterial filaments to cross the cell-cell boundary. These include an actin regulatory protein Ysc84 that is predicted to be secreted, an outer membrane phospholipase A_1, a patatin-like phospholipase A_2, and three secreted phospholipase C. We plan to knock out candidates for host cortical actin and membrane disruption to elucidate molecular mechanism of cell-cell crossing by *B. atropi* filaments. These results will further shed light on the mechanisms that an intracellular bacterial pathogen can employ for cell-cell spreading in an in vivo model.

1159B **Intracellular Bacterial Filamentation in *Oscheius tipulae* hints at an Evolutionarily Conserved Mechanism in the *Bordetella* Genus** Niklas G Perslow, Tuan D Tran, Robert J LuallenBiology, San Diego State University

Free-living nematodes serve as great models for understanding microbial infection, in part due to the capacity to observe infection phenotypes in live or intact animals. *Bordetella atropi* is a novel gram-negative bacterium isolated from the nematode *Oscheius tipulae* and is observed in vivo to utilize filamentation as a unique spreading mechanism. Bacterial filamentation is normally seen as a response to stress brought on by host defenses, environmental stressors, the presence of antibiotics, or DNA damaging mutagens. Canonically, filamentation by bacteria is induced through the SOS response due to extracellular stressors including host defense. The Bordetella genus in known for predominantly causing extracellular infection as coccobacilli. However, *B. atropi* diverges from the genus in its mode of infection, as it is a facultative intracellular pathogen that can spread from intestinal cell to intestinal cell through filamentation. Additionally, *B. atropi* utilizes nutrient sensing to initiate filamentation, as we isolated non-filamentous mutant strains that had loss-of-function alleles in a highly conserved metabolic pathway that antagonizes bacterial cell division in nutrient rich conditions. To understand the molecular mechanism of nutrient-induced filamentation we are studying the role of this metabolic pathway, the UDP-glucose pathway, in controlling *B. atropi* filamentation. We found that there may be a correlation between expression levels of one enzyme in the pathway, *gtaB,* and bacterial cell size in vitro. Additionally, we found that overexpression of *gtaB* in vivo leads to longer filaments in *O. tipulae.* Separately, we are investigating the evolutionary conservation of nutrient-induced filamentation across the Bordetella genus using *Bordetella avium* and *Bordetella bronchiseptica*. We have been able to induce filamentation in *B. avium* in vitro through switching the bacteria from standard growth media to enriched media. Additionally, we have created a non-filamentous mutant of *B. avium* using in vitro selection and will test if we hit mutations in the UDP-glucose pathway. Similar experiments will be conducted using *Bordetella bronchiseptica*. Overall, we aim to understand the mechanisms and evolutionary conservation of nutrient-induced filamentation across the *Bordetella* genus and determine if this mode for intracellular infection is conserved in other host species,

spanning from nematodes to birds to humans.

1160B **Understanding how microbial products may be influencing the RQ-dependent metabolism in *C. elegans*** Selin Ozgur, William Navarre, Andy Fraser Molecular Genetics, University of Toronto

The microbiota of the host play an important role in the well-being of every gut parasite. More importantly, the composition of host microbiota can influence parasitic helminth colonization and persistence within the host. Parasitic helminths need to be grown in mammalian models and can be hazardous to work with, however, the free-living nematode *C. elegans* shares a significant portion of its biology with parasitic helminths, and can be a convenient tool for high-throughput microbiota screens.

Soil transmitted helminths have a unique anaerobic metabolism that they share with *C. elegans* that is required for survival in hypoxic conditions, such as the gut. This metabolism is dependent on the electron carrier rhodoquinone (RQ) and is not shared with the hosts. In our lab, we use potassium cyanide (KCN), an inhibitor of Complex IV, to artificially incite the worms into switching to their RQ-dependent metabolism. In this study, our main goal is to carry out high-throughput microbiota screens on *C. elegans* to better understand the interplay between host, microbiota and parasite, and we are providing a fresh perspective by screening worms that have switched to their RQ-dependent metabolism which mimics the state parasitic helminths are in as they normally interact with gut microbiota.

So far, we have completed our first bacterial library screen, the *E. coli* standard reference (EcoR) library screen, and identified an *E. coli* isolate that impairs *C. elegans* from surviving in their RQ-dependent metabolic state. We have also identified several *C. elegans* hypoxia response mutants that are able to survive in their RQ-dependent state when they are fed our isolate of interest. We hypothesize that this *E.coli* isolate is able to stop the worms from activating their hypoxia response, which would give an advantage to the bacteria in hypoxic conditions such as the gut.

1161B **Branched chain fatty acids-rich diet promotes lipid droplet enlargement through SCAV-4/CD36 in *C. elegans*** Kam Ue Roy Li, Wing Ka Lo, Chenyin Wang, Chun Yin Lau, Chaogu Zheng School of Biological Sciences, The University of Hong Kong, Hong Kong

Lipid droplets (LDs) are conserved cellular organelles across various species, including *Caenorhabditis elegans*. Supersized LDs in *C. elegans* can be derived from dietary manipulation or genetic mutations. We found that a species of bacteria previously isolated from *C. elegans* natural habitat can induce enlarged LDs. Gas chromatography-Mass spectrometry (GC-MS) analysis showed that the bacteria contain almost exclusively monomethyl branched-chain fatty acids (mmBCFAs). When fed to the animals, the mmBCFAs derived from the bacterial diet accumulate in the triglycerides and phospholipids of the worms.

Through genetic studies, we found that the metabolism of mmBCFAs is regulated by the CD36 family scavenger receptor SCAV-4. We isolated a gain-of-function missense mutation in *scav-4* that further promoted LD enlargement under the mmBCFAs-rich bacterial diet. Interestingly, the deletion of *scav-4* led to decreased lipid accumulation and delayed development on both *E. coli* OP50 and the mmBCFA-rich natural bacteria diet. SCAV-4 is the homolog of CD36, which is a fatty acid transporter in human. However, its function in transporting mmBCFAs is not studied. We found that SCAV-4 is specifically expressed in the intestine and is localized to the apical membrane facing the intestinal lumen. Thus, we hypothesize that SCAV-4 is normally involved in transporting lipids into intestinal cells and the missense mutation we isolated may enhance the transport of mmBCFAs.

Moreover, transcriptomic analysis revealed that a fatty acid desaturase gene, *fat-7*, is up-regulated by the mmBCFA-rich bacteria diet. Deleting *fat-7* could abolish supersized LD induced by the natural bacteria, suggesting that *fat-7* is essential for LDs expansion induced by the mmBCFA-rich diet. FAT-7 is important for the synthesis of polyunsaturated fatty acids (PUFA) which could enhance LD expansion through rapid LD fusion of several smaller LDs, and we have observed increased occurrence with mmBCFA-rich diet. Combining with the known role of PUFAs in supporting SEIP-1-dependent LD budding process in the endoplasmic reticulum, we reasoned that mmBCFA-rich diet promotes LD expansion via both increased *de novo* synthesis and rapid LD fusions.

Our studies highlight the interaction between the dietary lipids from natural bacteria and the genetic context of the animals in the regulation of lipid metabolism, possibly providing a model for the influence of gut microbiome on the genetic predisposition to lipid disorders.

1162B **Characterization of the biochemical activity and tumor-promoting role of the dual protein methyltransferase METL-13/METTL13 in *Caenorhabditis elegans*** Melanie L. Engelfriet, Jędrzej M. Małecki, Anna F. Forsberg, Pål Ø. Falnes, Rafal CioskBiosciences, University of Oslo

The methyltransferase-like protein 13 (METTL13) methylates the eukaryotic elongation factor 1 alpha (eEF1A) on two locations:

the N-terminal amino group and lysine 55. The absence of this methylation leads to reduced protein synthesis and cell proliferation in human cancer cells. Previous studies showed that METTL13 is dispensable in non-transformed cells, making it potentially interesting for cancer therapy. However, METTL13 has not been examined yet in whole animals. Here, we used *C. elegans* as a simple model to assess the functions of METTL13. Using methyltransferase assays and mass spectrometry, we show that the *C. elegans* METTL13 (METL-13) methylates eEF1A (EEF-1A) in the same way as the human protein. Crucially, the cancer-promoting role of METL-13 is also conserved and depends on the methylation of EEF-1A, like in human cells. At the same time, METL-13 appears dispensable for animal growth and development. This makes *C. elegans* a convenient whole-animal model for studying METL13-dependent carcinogenesis without the complications of interfering with essential wild-type functions.

1163B **POT-3 preferentially binds the terminal DNA-repeat on the telomeric G-overhang** Xupeng Yu, Sean Gray, J Carlos Penedo, Helder C Ferreira University of St Andrews

Eukaryotic chromosomes typically end in 3‘ telomeric overhangs. The safeguarding of telomeric single-stranded DNA overhangs is carried out by factors related to the protection of telomeres 1 (POT1) protein in humans. Of the three POT1-like proteins in *Caenorhabditis elegans*, POT-3 was the only member thought to not play a role at telomeres. Here, we provide evidence that POT-3 is a *bona fide* telomere binding protein. Using a new loss-of-function mutant, we show that the absence of POT-3 causes telomere lengthening and increased levels of telomeric C-circles. We find that POT-3 directly binds the telomeric G-strand *in vitro* and map its minimal DNA binding site to the six-nucleotide motif, GCTTAG. Using fluorescence resonance energy transfer (FRET), we see that POT-3, in contrast to the closely related POT-2, prefers binding this repeat when it is immediately adjacent to the 3’ end of DNA. We are currently studying the effects of *pot-2* and *pot-3* mutation in the context of senescence. Our preliminary results show that the loss of POT-2 or POT-3 elongate the lifespan of *mrt-1* worms. Interestingly, *mrt-1; pot-3* strains show increased telomere heterogeneity compared to *mrt-1; pot-2*. These data indicate that POT-2 and POT-3 do not function redundantly with each other and highlights the rapid evolution and specialisation of telomere binding proteins.

1164B **Understanding How Germline Signals and Diet Interact to Impact *C. elegans* Lifespan** rhianne kelleher[1], luke reynoldson[1], hugo aguilaniu[2], andre pires da silva[11]School of Life Sciences, University of Warwick, [2]Serrapilheira Institute

Dietary restriction can improve health and increase lifespan across many different species. However, this increase in lifespan is often coupled with changes in reproductive function as resources are diverted from reproduction to somatic maintenance in times of nutrient scarcity.

The gonad of *Caenorhabditis elegans* (*C. elegans*) is involved in the regulation of longevity: extensions to lifespan can be induced via laser ablation of germline precursor cells, or by using germlineless mutants. However, this increase in lifespan is not solely due to sterility – instead this process requires multiple genes involved in nutrient-sensing, steroidal signalling, and longevity pathways. This would suggest that dietary signals, reproductive function, and longevity are linked.

It is well known that specific nutrients can modulate an organism’s health and lifespan. We present preliminary results suggesting that certain nutrients in combination with dietary restriction can drastically extend the lifespan of germlineless nematodes. This project thereby aims to identify the genetic factors that mediate the interplay between nutrient consumption, dietary restriction, and germline-mediated longevity.

1165B **Identification of natural variation in resistant to intracellular bacterial infection in the nematode *Oschieus tipulae*** Candyd Lace R Velasquez, Sandra Lee, Robert Luallen San Diego State University

Intracellular bacterial pathogens are able to infect host cells and utilize host machinery for their replication. Nematodes would make great model for understanding the mechanisms of intracellular bacterial infection and host immunity to these parasites. Through the use of genetic tools and manipulation, we can learn more about how the host cell is infected and how it responds to the infection itself. Here we have identified a nematode strain of *Oschieus tipulae* JU457 that is unable to be infected against an intracellular bacterium, *Bordetella atropi.* Normally, *B. atropi* can infect nematodes by spreading via filamentation through the intestine. We wanted to determine if there is natural variation in *O. tipulae* susceptibility to this intracellular bacterium. We screened multiple wild isolates of *O. tipulae* and observed that high variability in infection rates. Some *O. tipulae* strains showed a high infection rate, including wild type strain CEW1 and the cognate strain *B. atropi* was discovered in JU1501. By contrast, we found that some strains were highly resistant to infection, including the JU457. To validate these findings, infection was repeated using the strains CEW1, JU728, JU259, BA1009, and JU457 (all three considered to be more resistant). From this experiment, all worms, except for JU457 had 70% rate of infection or higher, whereas JU457 had less than a 4% infection rate.

In order to identify the regions that are responsible for JU457 resistancewe plan to utilize bulk segregant analysis . We have confirmed that JU457 males of *O. tipulae* can mate to the type strain CEW1. We have verified that JU457 resistance is recessive, as 100% of the F1 generation from this cross failed to survive *B. atropi* infection. Finally, we repeated this CEW1 x JU457 cross and

are currently testing the F2 generation to determine the level of *B. atropi* resistance among these animals. After this validation, we will use the selective pressure of *B. atropi* infection to select for resistant F2 progeny. Then, whole genome sequencing will be used to identify the genomic regions(s) associated with JU457 resistance to infection. In order to increase variability, worms may be allowed to self for 3 generations (F5 or F6) before sequencing. Mapping this region in JU457 will allow us to identify candidate loci that are associated with resistance. Then, we can utilize targeted CRISPR-Cas knockouts to identify the gene(s) conferring resistance to intracellular bacterial infection. Conversely, rather than identifying resistance alleles, we may instead identify susceptibility genes in CEW1 that lead to increased infection by *B. atropi*.

1166B **Gut microbiome alters bioenergetic pathway utilization impacting mitochondrial function and pollutant susceptibility** Christina Bergemann, Joel Meyer Duke University

The gut microbiome affects host health in numerous ways including influencing mitochondrial function and modulating xenobiotic toxicity. However, mechanisms through which the complex gut microbiota can alter mitochondrial function and susceptibility to mitochondrial toxicity are not well characterized. I aim to identify mechanisms by which gut microbiota impact mitochondria and if these interactions can modify susceptibility to chemical pollutants. Gut microbiota release metabolites that can influence mitochondrial function by altering bioenergetic pathways including the electron transport chain, fatty acid oxidation, and glycolysis. Reliance on varying pathways could impact sensitivity to mitochondrial toxicants. To explore this, I am using the model organism *Caenorhabditis elegans* and the microbiome kit CeMbio. I have selected six strains from CeMbio plus two laboratory standard bacteria, OP50 and HT115. Synchronized adult *C. elegans* grown on selected CeMbio strains had varying levels of steady-state ATP, with a 3.3-fold difference between the highest and lowest strains. Further, I found that nematodes grown on these strains varied in mitochondrial copy number, activation of mitochondrial stress pathways, and redox tone. Preliminary data has shown differences in sensitivity to the complex I inhibitor rotenone, the complex II inhibitor thenoyltrifluoroacetone, and the ATP synthase inhibitor, dicyclohexylcarbodiimide. Differences in chemical sensitivities indicate that nematodes grown on these bacteria vary in their reliance on glycolysis and electron donation from complexes I and II, possibly explaining varying levels of steady-state ATP. Overall, these data suggest that bacterial diets can have significant impacts on mitochondrial function through multiple mechanisms and that these changes can alter susceptibility to mitochondrial toxicants. Ongoing experiments include quantifying additional mitochondrial endpoints including respiration, as well as untargeted metabolomics to identify metabolites driving differences in bioenergetic pathway utilization.

1167B **Age dependent changes in the *C. elegans* gut microbiome and their significance** Rebecca Choi, Rahul Bodkhe, Barbara Pees, Dan Kim, Maureen Berg, David Monnin, Juhyun Cho, Vivek Narayan, Ethan Deller, Michael ShapiraIntegrative Biology, University of California Berkeley

The gut microbiome has been shown to play important roles in host function and health. Core microbiomes have been described for different species, and imbalances in these microbiomes, also known as dysbiosis, are associated with pathology. The gut microbiome also changes during aging, possibly due to the multi-tissue deterioration which includes metabolic shifts, dysregulated immunity, and disrupted epithelial barriers. Age-dependent changes in the gut microbiome have been reported in various organisms, but the relationship between the aging host and the gut microbiome is not well understood. Raising worms in natural-like microcosm environments or on two distinct communities of worm gut commensals revealed similar expansions in the relative abundance of *Enterobacteriaceae*, a family that is part of the worm core gut microbiome. One species within this family, *Enterobacter hormaechei,* normally an infection-protective commensal in young adults, became an exacerbating factor in aging worms. Its intestinal abundance, shown to be controlled by Sma/BMP signaling (which we found to decline in aging worms), increased in aging worms, and was associated with reduced infection resistance. Fortunately, these detrimental effects could be mitigated by competition with commensal communities, stressing the importance of maintaining a sufficient microbiome diversity to prevent detrimental blooms of some of its members.

1168B **Characterizing partners of Nuclear Hormone Receptor NHR-49 in starvation stress response and longevity** Glafira Ermakova, Kelsie Doering, Stefan Taubert 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.

1169B **Using *C. elegans* to decode the developmental origin of health and diseases** Yihan Wang, Yitian Tang, Weina Xu, Runshuai Zhang, Shenlu Qin, Lianfeng Wu School of Life Sciences, Westlake University

Environmental insults during early life may result in an increased risk of chronic disorders in late life, such as obesity and diabetes, the notion of which has been well known as the Developmental Origins of Health and Disease (DOHaD) hypothesis. However, the exact molecular machinery of DOHaD remains poorly understood, largely due to the lack of suitable model for studying this temporospatial phenomenon. *C. elegans* is a feasible model organism widely used for mechanistic studies of human diseases because of its short life cycle, ease of genetic manipulation, and highly conserved metabolic pathways with human. Our recent work, greatly supporting *C. elegans* as a valuable model for DOHaD basis study, revealed that early-life vitamin B12 deficiency leads to increased lipogenesis and lipid peroxidation, which in turn cause germline defects in adult *C. elegans* via ferroptosis (Cell Reports, 2022). In the current study, we sought to explore the genetic basis underlying the developmental programming of adulthood disorders using *C. elegans*. A custom RNAi library against 1013 genes that highly and specifically express in embryonic and early larva stages was created and applied in our DOHaD basis screen. The screen was set up by feeding P0 mothers with individual RNAi to knock down genes in the early life of their offspring and evaluating the offspring's fat level via Nile Red staining and fluorescence microscopy. One of our candidate genes with highest scores was *dohd-1* (developmental origins of health and disease gene 1), having a human orthologue annotated as a chromatin remodeling factor, knockdown of which in early life led to increased fat level in adulthood. To further prove that the long-lasting effect of *dohd-1* deficiency is truly derived from early-life interruption on the protein function, we constructed a *dohd-1* knock-in line with AID sequence to facilitate the inducible and reversible protein degradation exclusively in the embryonic stage. The embryonic degradation of DOHD-1 indeed consistently increased fat level in later life. Our ongoing efforts are aimed at elucidating the underlying mechanisms how the early-life DOHD-1 exerts its long-lasting impact on the fat metabolism in adulthood and what specific environmental insults in early life would program late-life lipid metabolism disorders through our newly defined DOHD-1 signaling.

1170B **The nuclear CUL-3 ubiquitin ligase adaptor SPOP-1 is a conserved regulator of proteotoxic stress in *C. elegans* neurons** Randall J Eck[1], Rebecca L Kow[1,2], Nicole F. Liachko[1,2], Brian C Kraemer[1,21]Department of Medicine, University of Washington, [2]Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System

The accumulation of misfolded proteins in neurons during aging contributes to a progressive decline in neuron function in *C. elegans*. In humans, neuron death in age-related neurodegenerative diseases is driven by pathological protein aggregation and neurotoxicity. We find that tau toxicity depends on SPOP (speckle-type POZ protein) in a *Caenorhabditis elegans* model of tauopathy, characteristic of Alzheimer's disease. SPOP is a conserved nuclear adaptor protein for the RING E3 ubiquitin ligase Cullin-3 (CUL3), selecting proteins for degradation in the ubiquitin proteasome system (UPS) and localizing to nuclear speckles (NS). Loss of function mutations in the *C. elegans spop-1* reduce the accumulation of insoluble and phosphorylated tau, improve behavioral deficits, extend lifespan, and prevent neurodegeneration in *C. elegans* expressing mutant and wildtype human tau in neurons. Previous research in *C. elegans* identified SPOP as a regulator of C9orf72 dipeptide repeat toxicity, a familial form of the neurodegenerative disease amyotrophic lateral sclerosis (ALS). In that case, SPOP/CUL3 substrate BRD2/3/4, *bet-1* in *C. elegans*, was required for suppression. In tauopathy, we find *bet-1* is not required. Instead, we propose that SPOP-CUL3 UPS activity and SPOP liquid-liquid phase separation (LLPS)/NS localization contribute to the underlying mechanisms of tauopathy. We will continue to explore the overlapping and distinct mechanisms by which SPOP modifies protein aggregation and neurodegeneration across several diverse proteinopathies. Altogether, our results suggest SPOP-1 is a common regulator of proteotoxicity in *C. elegans* neurons. Further study of SPOP function has novel and compelling potential to uncover molecular mechanisms underlying proteotoxic stress in diverse disorders and may inform future therapeutic development.

1171B **No rest for the weary: a comparative analysis of the molecular and physiological consequences of SKN-1 activation without an off-switch** Carmen M. Ramos[1], Sean P. Curran[21]Molecular and Computational Biology, University of Southern California, [2]Leonard Davis School of Gerontology, University of Southern California

Molecular homeostats play essential roles across all levels of biological organization, from molecules to organism, to ensure a return to normal function after responding to abnormal internal and environmental events. SKN-1 is an evolutionarily conserved cytoprotective transcription factor that is integral for the maintenance of cellular homeostasis upon exposure to a variety of stress conditions. Despite the essentiality of turning on SKN-1/Nrf2 in response to exogenous and endogenous stress, animals with chronic activation of SKN-1 display premature loss of health with age, and ultimately, diminished lifespan. Previous genetic models of constitutive SKN-1 activation include gain-of-function alleles of *skn-1* and loss-of-function alleles of *wdr-23* that impede the turnover of SKN-1 by the ubiquitin proteasome. Here we define a novel gain-of-function mutation in the *xrep-4* locus that results in constitutive activation of SKN-1 in the absence of stress. Although each of these genetic mutations results in

continuously unregulated transcriptional output from SKN-1, the physiological consequences of each model on development, stress resistance, reproduction, lipid homeostasis, and lifespan are distinct. Here we provide a comprehensive assessment of the differential healthspan impact across multiple models of constitutive SKN-1 activation. Although our results reveal the universal need to reign in the uncontrolled activity of cytoprotective transcription factors, we also define the unique signatures of each model of constitutive SKN-1 activation, which provides innovative solutions for the design of molecular "off-switches" of unregulated transcription output.

1172B **Neuronal HSF-1 cell-nonautonomously regulates longevity via intestinal serotonin and octopamine receptors and DAF-16 activity.** Yu-Jhen Chen[1], Yi-Hao Huang[1], Yu-Hao Chang[1], Tsui-Ting Ching[2], Ao-Lin Hsu[1,31]Biochemistry & Molecular Biology, National Yang Ming Chiao Tung University, [2]Biopharmaceutics Sciences, National Yang Ming Chiao Tung University, [3]Internal Medicine-Geriatric & Palliative Medicine, University of Michigan

Inter-tissue communications are integral to organismal aging, orchestrating a system-wide aging process. Transcription factors such as the FOXO transcription factor DAF-16 and heat shock transcription factor HSF-1 are known to play pivotal and conserved roles in regulating aging. DAF-16/FOXO were shown to act in the intestinal cells to mediate the longevity effects of the IIS (Insulin/IGF-1-like signaling) pathway mutants. Interestingly, recent studies suggested that neuronal *hsf-1* functions cell non-autonomously to regulate longevity via activation of intestinal DAF-16. However, the molecular mechanisms by which neuronal HSF-1 modulates intestinal DAF-16 activity remains unclear. In this study, we found that activation of HSF-1 only in the cholinergic neurons is sufficient to extend lifespan. While the activity of intestinal DAF-16 is required for neuronal HSF-1 to modulates lifespan, the nuclear translocation of DAF-16 is not. Furthermore, we found that both the serotonin and octopamine receptors expressed in the intestinal cells appear to be required to transduce the lifespan signals generate from HSF-1 in the cholinergic neurons, suggesting a more complicated neuronal circuit may be involved. Our studies further elucidated the potential cellular and molecular mechanisms by which neurons communicate to intestinal cells to orchestrate a synchronized aging process.

1173B **High glucose diet shortens lifespan by modulating SET-2 and HLH-30/TFEB activity.** Chun-An Lin[1], Feng-Yung Wang[2], Yun-Chang Wu[1], Ao-Lin Hsu[2], Tsui-Ting Ching[11]Biopharmaceutical Sciences, National Yang Ming Chiao Tung University, [2]Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University

Excess sugar consumption is associated with the development of various pathological conditions, such as diabetes and cardiovascular diseases. In *C. elegans,* high-glucose diet (HGD) increases fat accumulation and shortens lifespan. Here, we found that HGD attenuates the expression of several autophagy genes (e.g. *lgg-1*) and suppresses autophagy activity. We have previously reported that SET-2-mediated H3K4me3 methylation suppresses the transcriptional activity of HLH-30/TFEB. Intriguingly, in our recent studies, we found that HGD could elevate H3K4me3 level and reduce HLH-30/TFEB levels, which consequently causes the reduction of autophagy genes. We also found that HGD could not further shorten the lifespan of *hlh-30* null mutants*,* suggesting that *hlh-30* is required for HGD-induced lifespan shortening phenotype. Moreover, HGD fails to shorten the lifespan of *set-2* or *sams-1* mutants, indicating that HGD-induced lifespan shortening is dependent on increased H3K4me3 level. Together, we hypothesize that nutrient-mediated H3K4me3 changes might regulate HLH-30/TFEB activity to modulate autophagy functions, which ultimately influence the rate of aging and longevity in *C. elegans*.

1174B **Investigating the heterochromatin model of *C. elegans* aging using ChromID proximity labeling** Valeryia Aksianiuk[1,2], William Smith[1,2], Devanarayanan Siva Sankar[3], Joern Dengjel[3,4], Rodrigo Villaseñor[5], Tuncay Baubec[6], Peter Meister[11]Institute of Cell Biology, University of Bern, [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, [3]Department of Biology, University of Fribourg, [4]Metabolomics and Proteomics Platform, University of Fribourg, [5]Molecular Biology Division, Biomedical Center Munich, [6]Division of Genome Biology and Epigenetics, Utrecht University

Loss of heterochromatin, a transcriptionally repressive chromatin state, is believed to be a major contributor to organismal aging. Heterochromatin is established and maintained through post-translational modifications of histone tails, which increase chromatin compaction and create transcriptionally inaccessible chromatin regions anchored to the nuclear periphery. This is accomplished by trimethylation of the ninth and twenty-seventh lysines on histone type 3 (H3K9me3 and H3K27me3), which are hallmarks of heterochromatin. Constitutive heterochromatin is characterized by H3K9me3 and typically silences satellite repeats and transposable elements in the genome while localizing DNA to the nuclear periphery. On the other hand, facultative heterochromatin is marked by H3K27me3, transiently silences genes, varies during development, and silences some tissue-specific promoters. Older nematodes display age-related nuclear changes, including heterochromatin loss and the loss of proteins that interact with heterochromatin. It is postulated that the dysregulation of heterochromatin and its protein interactome leads to widespread derepression of silenced genes, which may drive aging phenotypes and death in both nematodes and mammals. However, the underlying mechanisms are not fully understood. To identify candidate proteins that may play a role in this proposed epigenetic aging mechanism, we utilize ChromID, an *in vivo* proximity labeling technique specific to chromatin marks. Temporal control of the ChromID construct makes it possible to identify changes in the heterochromatin interactome

throughout *C. elegans* lifespan. These ChromID hits will form the basis of experiments that seek to characterize the functional role of the heterochromatin interactome in *C. elegans* lifespan.

1175B **A screen to define the physiological roles of polyunsaturated fatty acids in *C. elegans*** Delaney Kaper, Uros Radovic, Marc Pilon Chemistry and Molecular Biology, University of Gothenburg

Polyunsaturated fatty acids (PUFA) play an essential role in the metabolism, membrane homeostasis, and development of living organisms. Lack of PUFAs leads to multiple defects but it is difficult which are primary consequences of PUFA deficiency, and which are secondary effects. To investigate the function of and role of PUFAs, we are studying a near-null allele of the worm's single FAT-2 Δ12 desaturase that converts a MUFA (18:1n9, oleic acid) into a PUFA (18:2n6, linoleic acid). This *fat-2(wa17)* allele was isolated and previously described in Watts and Browse, PNAS 99:5854; 2002. Additional characterization of the phenotype confirmed or revealed several defects in the mutant worms, including a slower growth rate, locomotion defects, intolerance to cold and a rigid plasma membrane (assessed using in vivo fluorescence recovery after photobleaching, i.e. FRAP). After testing several unsaturated fatty acids (18:1n9, oleic acid (OA); 18:2n6, linoleic acid (LA); 20:5n-3, eicosapentaenoic acid (EPA) and 22:6n3 docosahexaenoic acid (DHA)) and a detergent (NP40) for their ability to suppress the *fat-2(wa17)* phenotypes, we found that only LA, DHA and EPA were efficacious. Like the *fat-2(wa17)* mutant, null *paqr-2* mutants have rigid membranes and multiple defects. We previously identified several mutations that act as suppressors of the *paqr-2* by promoting either fatty acid desaturation (e.g. *mdt-15(et14), nhr-49(et8)*) or incorporation of PUFA into phospholipids (e.g. *acs-13(et54)*and *fld-1(et46)*). We found that these *paqr-2* suppressors act as extremely weak *fat-2(wa17)* suppressors, which is not surprising considering that the *fat-2(wa17)* is already able to produce MUFA and essentially lacks PUFA that could be incorporated into phospholipids. We have performed a forward genetic screen to identity more potent *fat-2(wa17)* suppressors in the hope that this will lead to the identification of specific pathways or cellular processes that are particularly sensitive to PUFA depletion, or of specific mechanisms that compensate for PUFA shortage. Ten *fat-2(wa17)* suppressors have been isolated, outcrossed 6X and their genomes sequenced. Initial analysis of the sequenced genomes shows that some of these mutants acquired mutations within *fat-2* itself, confirming that the screen was successful.

1176B **Biological mechanisms of age-related alpha-synuclein toxicity and phase behaviour** Suzanne Couzijn, Eugenia Goya, Renée I Seinstra, Celine N Martineau, Inge Werkman, Ellen A.A. NollenEuropean Research Institute for the Biology of Aging

Protein homeostasis is important to maintain a stable and functional proteome over time. Even though many cellular pathways are involved in maintaining a stable proteome, the capacity of these pathways declines with aging. Aggregation-prone proteins harbouring intrinsically disordered regions or low-complexity domains can undergo the phenomenon of liquid-liquid phase separation in which protein droplets are de-mixed from the solution. During aging, these droplet inclusions can transition into solid aggregates. This process is thought to contribute to proteotoxicity in age-related neurodegenerative diseases such as Parkinson's disease. The exact biological mechanisms, however, that drive protein toxicity are still poorly understood. Here we aim to identify biological processes involved in the toxicity of alpha-synuclein, involved in Parkinson's disease, using a *Caenorhabditis elegans* as a model. By genome-wide RNAi screening and automated motility tracking, we have identified genes that reduce the toxicity of alpha-synuclein expressed in body-wall muscle cells. To further unravel the cellular mechanisms involved in toxicity, we developed a *Caenorhabditis elegans* model expressing alpha-synuclein in its dopaminergic neurons. Dopaminergic expression of alpha-synuclein seems to result in an age-related increase in inclusions. By combining the results from the genome-wide screen and our neuronal alpha-synuclein *C. elegans* model, we hope to further unravel the cellular mechanisms underlying protein toxicity and gain new insights into mechanisms affecting protein phase transitions in aging and age-related diseases.

1177B **More than a neurotrophic factor: MANF-1 regulates proteostasis & autophagic flux** Shane Kevin K. B. Taylor, Bhagwati P. Gupta Biology, McMaster University

Age-related diseases such as Parkinson's Disease (PD) and Huntington's Disease (HD) are associated with detrimental activation of the stress response and toxic protein aggregation. Neurotrophic factors (NTFs) are promising therapeutic candidates for the treatment of such diseases because of their potential roles in promoting neuronal health and survival. Interestingly, the *C. elegans* genome encodes only one NTF, *manf-1,* a homolog of the **M**esencephalic **A**strocyte Derived **N**eurotrophic **F**actor (MANF). MANF is conserved in eukaryotes and plays an essential role in neuroprotection. Previously, we reported that *manf-1* expression declines with age and mutations in *manf-1* cause a range of defects including increased stress sensitivity, protein aggregation, and age-dependent dopaminergic (DA) neurodegeneration.

Our recent work has revealed that *manf-1* overexpression activates the endoplasmic reticulum- unfolded protein response transcription factor *xbp-1*, promotes DA neuron protection, reduces protein aggregation in models of PD and HD, and extends the lifespan of animals. We also observed that *manf-1* promotes nuclear localization of the autophagy transcription factor *hlh-30/*TFEB. This suggests that *manf-1* induces autophagy to elicit its cytoprotective role. In support of this, we see that *manf-*

1 overexpression caused a reduction in the autophagosome receptor SQST-1/p62 and a decrease in LGG-1 positive autophagosome-related puncta which is indicative of improved autophagic flux. Collectively, our work provides a new understanding of MANF-1 as more than a neurotrophic factor, but a cytoprotective protein with diverse function through the clearance of protein aggregates and improved autophagic flux.

1178B **Are levels of autophagy increased or decreased in long-lived *daf-2* insulin/IGF-1 mutants?** Hannah Chapman[1], Kuei Ching Hsiung[2], David Gems[21]Department of Genetics, Evolution and Environment, UCL - Institute of Healthy Ageing, [2]UCL - Institute of Healthy Ageing

According to one theory, molecular damage is the principal cause of aging, while somatic maintenance mechanisms act against damage accumulation and aging. One such maintenance mechanism is autophagy, which can remove damaged cellular constituents. Thus, autophagy is considered a likely mechanism protecting against aging. Various findings support the view that autophagy levels are elevated in *daf-2* insulin/IGF-1 receptor mutants in *C. elegans*, and that autophagy contributes to their extended lifespan.

We have been investigating the causes of intestinal atrophy, a major senescent pathology in *C. elegans*. Our previous evidence suggested that gut atrophy is the result of the intestine consuming its own biomass to support yolk synthesis in post-reproductive hermaphrodites [1], an example of a programmatic mechanism of aging [2]. This yolk is vented via the vulva after reproduction and can support larval growth [3]. Intestinal atrophy is suppressed by inhibition of autophagy, and greatly reduced in *daf-2* mutants [1,4], hinting that autophagy might be *decreased* in *daf-2* mutants. Consistent with this, overall protein turnover is reduced in *daf-2* mutants [5,6]. A major limitation of current research is the difficulty in assaying autophagy reliably, resulting in incongruities between published studies.

To try to resolve what seems to be a discrepancy about the relationship between IIS, autophagy, and aging in *C. elegans* we have revisited the effects of knockdown of genes encoding the machinery of autophagy on pathology and aging, taking into consideration issues of timing and severity of knockdown, *daf-2* allele class, temperature, bacterial status, and other issues. We are also working with several autophagic flux protocols to assess how levels of autophagy change with genotype and age.

Our interim data will be described.

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1179B **The chaperone HSP110 as *in vivo* modulator of protein aggregation in Alzheimer's disease** Sabrina Montresor[1], Franziska Hirsch[1], Maria Lucia Pigazzini[2], Paul Spellerberg[1], Natascha Jacob[1], Yara Ehlert[1], Lukas Basilicata[1], Janine Kirstein[1,31]University of Bremen, [2]Leibniz Institute of Molecular Pharmacology, [3]Leibniz Institute on Aging – Fritz Lipmann Institute

Alzheimer's disease (AD) is characterized by the accumulation of Tau tangles and amyloid-beta (Aβ) plaques. Molecular chaperones are crucial in maintaining a functional proteome and have the potential to ameliorate neurodegenerative diseases. However, chaperones are double-edged swords. They can prevent the accumulation of toxic amyloid protein aggregates by suppressing their aggregation, but also enhance toxicity by releasing seeding competent protein species during disaggregation. A trimeric chaperone complex (HSP70, DNAJB1 and HSP110 in human) has the ability to suppress and disaggregate HTT, α-synuclein and Tau that are associated with Huntington's, Parkinson's and Alzheimer´s diseases, respectively. This complex suppresses Aβ and disaggregates Tau *in vitro*, but thus far *in vivo* data are missing. Yet, *in vivo* data are crucial to gain insight into the detrimental vs. beneficial potential of the chaperone machinery for the physiology of the organism.

HSP110 is an essential and abundant chaperone that acts as nucleotide exchange factor (NEF) in the Hsp70 chaperone cycle. It has been speculated that HSP110 exhibits additional chaperone activities besides its role as NEF and was hence here studied for its role in modulating protein aggregation in AD pathology *in vivo*. Our lab has established novel pan-neuronal Aβ and Tau *C. elegans* models. To assess the role of HSP110, we have generated GFP-tagged pan-neuronal *hsp-110* strain that leads to 20% overexpression. To complement the overexpression approach, *hsp-110* was depleted by RNAi. A knockout of *hsp-110* is lethal and further suggests that it is involved in critical protein folding processes. Consequently, it is not surprising that modulation of HSP-110, either overexpression or depletion, causes pleiotropic defects in growth and development. Nevertheless, we could also show that neuronal overexpression of *hsp-110::gfp* leads to an increased Aβ aggregation as assessed by fluorescence lifetime imaging. Thus, HSP-110 is a modulator of Aβ aggregation and pathology and its overexpression exacerbates the pathology as previously shown for α-synuclein. We are now studying the mechanistic underpinnings how HSP-110 favours Aβ aggregation. It has not yet been demonstrated that Aβ plaques can be disaggregated, but it is possible that HSP-110 is involved in this process, releasing seeding competent Aβ species that in turn facilitate Aβ aggregation. The novel Tau *C. elegans* model will reveal whether

the same mechanism applies to Tau or is unique for Aβ.

1180B **The dsRNA-binding protein RDE-4 regulates DAF-16 tissue localization and transgenerational longevity** Thomas Liontis, Andrés R Mansisidor, Jeeya Yogesh Patel, Masa Khairi, Alla Grishok Boston University Chobanian & Avedisian School of Medicine, Department of Biochemistry, Boston, MA 02118

Pioneering *C. elegans* work established the key role of the transcription factor DAF-16/FOXO in activating longevity-promoting metabolic pathways and the importance of conserved insulin/insulin-like growth factor (IGF)-1 signaling (IIS) in negatively regulating DAF-16. While the roles of microRNAs in reducing and promoting lifespan have been documented in *C. elegans* and other organisms, the contribution of endogenous dsRNA-derived small RNAs is less clear. RNA interference pathways involve binding of exogenous or endogenous dsRNAs to RDE-4, which directs them to Dicer for cleavage. Primary siRNAs bind to Argonautes such as RDE-1. After outcrossing all mutant strains 6 times to wild-type (N2), we find that *rde-4(ne301)* and *rde-1(ne219)* extend lifespan by 16 % and 8 %, respectively. Both mutations in the RNAi pathway similarly extend lifespan in the already long-lived reduced IIS mutant *age-1(hx546)*. Given that *age-1(hx546)* is a partial loss-of-function, this does not rule out RNAi and reduced IIS acting in the same pathway to extend lifespan. Indeed, the increased lifespan of *age-1; rde-4* double mutants is completely dependent on DAF-16. Interestingly, the life-extending effect of the *age-1(-/-); rde-4(-/-)* mutant worms persisted in 3rd and 4th-generation *age-1(-/-); rde-4(+/+)* descendants, consistent with possible epigenetic inheritance of the phenotype. We observed tissue-specific changes in *Pdaf-16::DAF-16::GFP* expression in *rde-4* mutants: elevated expression in the intestine and reduced expression in other tissues, such as muscle and epidermis. Consistently, the DAF-16 target *sod-3* is upregulated in the intestine of *rde-4* mutants. This suggests that endogenous siRNAs regulate DAF-16 localization and activity in a tissue-specific manner, with some siRNAs promoting DAF-16 and others inhibiting it. We performed small RNA-sequencing and evaluated primary siRNA landscapes in wild-type, *age-1*, *rde-4* and *age-1; rde-4* mutants and found a distinct group of siRNAs elevated in *age-1(hx546)* in an RDE-4-dependent manner. These siRNAs predominantly target nuclear-encoded and mitochondrial-encoded genes coding for the mitochondrial electron transport chain. We aim to uncover how endogenous siRNAs are regulating DAF-16 tissue-specific localization and activation. In addition, we will investigate whether endogenous siRNAs are regulating mitochondrial morphology and function and whether this could explain *rde-4(-)* longevity.

1181B **efk-1/eEF2K mediates mitochondrial function and oxidative damage repair to promote L1 starvation** Junran Yan[1,2,3], Forum Bhanshali[3], Tsultrim Mendenhall[4], Jessica Hartman[4], Stefan Taubert[2,3,51]Cell and Developmental Biology, University of British Columbia, [2]British Columbia Children's Hospital Research Institute, [3]Centre for Molecular Medicine and Therapeutics, University of British Columbia, [4]Department of Biochemistry and Molecular Biology, Medical University of South Carolina, [5]Department of Medical Genetics, University of British Columbia

During starvation, cells protect themselves by shutting down energy-demanding processes such as protein synthesis. Eukaryotic elongation factor 2 kinase (eEF2K) is a conserved kinase that responds to starvation by phosphorylating and inactivating eEF2, the rate-limiting driver of translation elongation, thus conserving energy for survival. The *C. elegans* eEF2K ortholog *efk-1* phosphorylates its target EEF-2/eEF2 on a conserved site and is essential for L1 starvation survival. However, it is unclear how *efk-1* ultimately promotes starvation survival. We identified two TFs that function in the *efk-1* pathway, ZIP-2/bZIP and CEP-1/p53, which upregulate transcription of DNA repair genes in starvation, such as nucleotide excision repair (NER) and base excision repair (BER) genes. Consistently, we confirmed by functional studies that several NER and BER genes are involved in *efk-1* mediated starvation resistance using the population starvation survival assay. Notably, exposure to starvation increases resistance to UV-induced DNA damage in wildtype animals, but not the *efk-1* mutant. These suggest that *efk-1* is required to activate DNA repair during starvation to promote genome integrity. Next, we asked if oxidative stress, specifically mitochondrial ROS, is a major source of starvation-induced DNA damage. Indeed, *efk-1* mutants accumulate excess ROS during starvation, and antioxidant supplementation is sufficient to rescue their starvation survival defect to WT levels. Supplementation with mitochondrially targeted antioxidant mitoquinone (MitoQ) partially rescued the survival defect of *efk-1* mutants, indicating that *efk-1* mutants suffer from a detrimental accumulation of ROS produced by the mitochondria. Furthermore, we also observed higher respiration rates in the *efk-1* mutant during starvation, which may explain the increased ROS. Finally, we asked whether *efk-1* regulates starvation survival via its canonical role of inhibiting EEF-2 mediated translation elongation. Surprisingly, unlike in mammalian models, starvation in *C. elegans* does not increase EEF-2 phosphorylation, and blocking translation elongation via cycloheximide fails to rescue starvation survival defects caused by *efk-1* loss. Taken together, we propose a model where *efk-1* promotes starvation survival via a translation elongation-independent pathway that involves preserving mitochondrial function, preventing accumulation of mitochondrial ROS and promoting repair of oxidative DNA damage.

1182B **Identification and characterization of host genes that *Stenotrophomonas maltophilia* targets to evade host insulin-like DAF-2/16 pathway defenses** Sara M. Hopkins, Leah J. Radeke, Michael A. Herman School of Biological Sciences, University of Nebraska-Lincoln

The bacterivorous nematode *Caenorhabditis elegans* has been developed as a 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.,* 2016). Thus, the study of this interaction has both medical and ecological relevance. We have previously shown that many of the *C. elegans* conserved innate immune 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. This finding suggests that pathogenic stains of *S. maltophilia* evade the pathogen resistance conferred by activation of the DAF-2/16 pathway. We used transcriptional profiling in *wild-type* and *daf-2* mutants to identify candidate *C. elegans* genes that *S. maltophilia* may target to defeat host defenses. To this end, we have identified 88 genes that are significantly differentially expressed in the absence of *daf-2* function upon exposure to *S. maltophilia.* We hypothesize that *S. maltophilia* may interfere with the function of expressed genes, and these candidate target genes will be contained within this subset. We also hypothesize that *S. maltophilia* may prevent the transcription of genes, and such candidate target genes will not be contained within this subset of differentially expressed genes. Regardless of the *S. maltophilia* mechanism of pathogenicity, we hypothesize that candidate target genes will be differentially expressed in response to other pathogens that are susceptible to DAF-2/16 pathway defenses and regulated by the DAF-16 transcription factor. These candidate gene criteria were used in conjunction with connectivity within a gene network model to select candidate target genes for functional analysis. We expect that candidate *S. maltophilia* target genes should be required for *daf-2*-mediated lifespan extension in the absence of the pathogen. To test this hypothesis, we are using RNA-mediated interference to knock-down candidate target genes in both *wild-type* and *daf-2* mutant backgrounds and evaluating the effect on lifespan. Future characterization of these candidate target genes may help us elucidate the underlying mechanisms that enable pathogenic *S. maltophilia* to defeat the nematode's innate immune responses.

1183B **Virgin vs Mated Worms, *a metabolomics approach*** Diana C Fajardo Palomino[1], Jintao Lu[2], Bennett W Fox[1], Douglas S Portman[3], Frank C Schroeder[11]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, [2]School of Life Sciences, Xiamen University, [3]Department of Biomedical Genetics, University of Rochester

Several studies have shown that *C. elegans* hermaphrodite lifespan is reduced by males, a phenomenon known as "male-induced demise" (MID), either by direct sexual interaction (mating)[1], or indirectly via exposure to compounds secreted by males[2]. Although male-derived compounds have been identified that accelerate hermaphrodite aging[3,4], the biochemical mechanisms that underlie the effects of mating remain unclear.

To address this question, we performed comparative metabolomic analysis of mated and virgin hermaphrodites, which revealed a stark increase in the abundance of several male germline-derived compounds in mated hermaphrodites. For example, nacq#1, an unusual N-acylated glutamine derivative that shortens hermaphrodite lifespan[4], as well as a new series of unusual β-methyl substituted fatty acids[3] that are derived from a novel NHR-49 agonist, are enriched in the mated hermaphrodites. In contrast, the abundance of the ascaroside ascr#10, which is also lifespan shortening but produced in the male intestine, not the germline[3] , was not affected by mating. These results suggest that Male germline-derived compounds are transferred during mating and implicate such transfer as a potential mechanism of MID.

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1184B **Maternal aging orchestrates offspring fat outcomes** Runshuai Zhang, Lianfeng Wu

School of Life Sciences, Westlake University

Obesity has increasingly become a worldwide major health problem. Growing evidence from epidemiological studies suggest that maternal status during pregnancy, such as maternal nutrition and mothers' ages, plays a critical role in programming offspring adulthood health outcomes. However, whether and how such maternal influences affect adult fat deposit remains largely unknown. Using the short-lived and genetically tractable nematode *C. elegans*, we recently discovered that offspring born to old mothers tend to have larger adulthood body size, more vulnerable behavior upon temperature challenges, better chemotactic responses and lower fat level than those born to young mothers, among which the body size formation is mainly regulated by the mitochondria-AMPK-TGFβ signaling axis (In revision in *Cell Research* at the moment of this abstract submission). Interestingly, we noticed that adult fat level has a robust and significant correlation with maternal aging. To further explore how maternal

aging programs offspring adult fat deposit, we first compared the fat level of offspring with gene mutations in mitochondrial electronic respiratory chain (ETC) or relative metabolic pathways. Our preliminary data showed that mutation of *nuo-6* (gene of complex I of mitochondrial ETC) or *daf-16* (a key transcriptional factor of insulin signaling pathway) can block the decreased fat phenotype in offspring of aged mothers, indicating a dependency of the mitochondria-insulin signaling axis for maternal age-mediated offspring adult fat outcomes. Our further goal is to reveal how the aged mitochondria inherited from aging mothers connect to insulin signaling in programming offspring fat outcome formation, which may provide novel options or targets for future anti-obesity therapies.

1185B **Meiotic dysfunction accelerates somatic aging in *Caenorhabditis elegans*** Arjumand Ghazi[1], Julia Loose[2], Francis RG Amrit[1], Judith Yanowitz[31]University of Pittsburgh School of Medicine, [2]University of Pittsburgh, [3]Obstetrics & Gynecology, University of Pittsburgh School of Medicine

An expanding body of evidence, from studies in model organisms to human clinical data, reveals that reproductive health influences organismal aging. However, the impact of germline integrity on somatic aging is poorly understood. Moreover, assessing the causal relationship of such an impact is challenging to address in human and verte-brate models. Here, we demonstrate that disruption of meiosis, a germline restricted process, shortened lifespan, impaired individual aspects of healthspan, and acceler-ated somatic aging in *Caenorhabditis elegans*. Young meiotic mutants exhibited tran-scriptional profiles that showed remarkable overlap with the transcriptomes of old worms and shared similarities with transcriptomes of aging human tissues as well. We found that meiosis dysfunction caused increased expression of functionally relevant longevity determinants whose inactivation enhanced the lifespan of normal animals. Further, meiotic mutants manifested destabilized protein homeostasis and enhanced proteasomal activity partially rescued the associated lifespan defects. Our study dem-onstrates a role for meiotic integrity in controlling somatic aging and reveals proteo-stasis control as a potential mechanism through which germline status impacts overall organismal health.

1186B **Post-transcriptional induction of fatty acid biosynthesis enables biguanide-mediated lifespan extension in *Caenorhabditis elegans*** Fasih M Ahsan[1,2], Armen I Yerevanian[1,3], Jose Aceves-Salvador[2], Alexander A Soukas[1,2,3,41]Department of Medicine, Diabetes Unit, and Center for Genomic Medicine, Massachusetts General Hospital, [2]Program in Biological and Biomedical Sciences, Harvard Medical School, [3]Department of Medicine, Harvard Medical School, [4]Broad Institute of Harvard and MIT

Metformin, the world's most prescribed anti-diabetic therapeutic, strikingly extends organismal lifespan in both vertebrate and invertebrate models. While mitochondrial complex I may represent a major molecular target of the drug, downstream mechanisms that link metformin's mitochondrial effects to extended lifespan are incompletely understood. Given the safety, efficacy, and well-tolerated nature of metformin, there is an urgent need to identify how metformin and related biguanides exert their favorable pro-longevity effects, to illuminate novel geroprotective targets and heretofore underappreciated mechanisms that modulate aging. Here, we perform a genome-wide screen for genetic regulators of metformin-mediated growth restriction in *C. elegans*, revealing that dampened cytosolic translation initiation partly mediates the drug's favorable effects on growth and lifespan. Profiling of mRNA species associated with actively translating ribosomal populations in animals treated with the sister biguanide phenformin show a strong reduction in translation efficiency of mitochondrial complex IV and heterogenous cytosolic ribosomal transcripts. Despite this widespread loss of translation efficiency, we identify protected mRNA translation of *de novo* lipogenic enzymes, concomitant with a decrease in translation efficiency of fatty acid desaturation and elongation transcripts during biguanide treatment. Critically, post-developmental inactivation of these lipogenic genes results in a complete abrogation of biguanide-mediated lifespan extension, resulting from a synergetic depletion of fatty acid oxidation activity and somatic lipid stores. In summary, we leverage reverse genetic, translatomic, and functional genomic strategies to discover a signaling axis connecting biguanide-mediated mitochondrial dysfunction to inhibition of cytosolic translation initiation, resulting in rewiring of lipid metabolism and fatty acid β-oxidation to improve proteostasis and extend organismal lifespan and healthspan.

1187B **Modeling Charcot-Marie-Tooth disease in *C. elegans*** Constantin Bretonneau[1,2], Claudia Maios[2], Éric Samarut[2,3], Alex Parker[2,31]Neuroscience, University of Montreal, [2]The University of Montreal Hospital Research Centre (CRCHUM), [3]University of Montreal

Charcot-Marie-Tooth disease (CMT) is a group of inherited disorders that affect the peripheral nerves, causing muscle weakness and atrophy, as well as sensory loss. CMT4J is a rare subtype of CMT caused by compound heterozygous mutations in the *FIG4* gene, which encodes a phosphoinositide phosphatase. *FIG4* pathogenic variants are also linked to Yunis-Varon Syndrome, ALS and, Polymicrogyria. The FIG4 protein forms a complex with PIKfyve and VAC14 that regulates levels of the lipid signaling molecules PI(3)P and PI(3,5)P2 which, in turn play a critical role in many cellular processes such as membrane homeostasis, exocytosis, stress signaling, and endolysosome acidification. How CMT4J-specific mutations in *FIG4* affect these canonical and, yet unexplored non-canonical pathways is still largely unknown.

We hypothesize that generating accurate genetic models of CMT4J carrying relevant *FIG4* mutations will lead to the discovery of actionable pathogenic mechanisms to accelerate drug and biomarker breakthroughs.

The ortholog of *FIG4* in *C. elegans* is *figo-1* and using the CRISPR/Cas9 technology, we established two CMT4J models: A null mutation strain and one with a knock-in I34T mutation (corresponding to patient I41T). Preliminary characterization indicates that null mutants and I34T mutants exhibit distinct CMT4J features. While FIGO-1 and its complex are localized at the membrane of endolysosomes, we found that null mutants display characteristic enlarged vacuoles, and that I34T mutants do not. Interestingly, in addition to severe motility defects linked to neurodegeneration and body-wall muscle defects, we observed that I34T mutants have altered lysosomal functions independent of vacuole size.

Our goal is to continue exploring the molecular basis of CMT4J and the pathogenicity of the FIG4(I41T) mutation.

1188B **Computational approach to identify potential genetic targets in a *C. elegans* model of glycogen storage disease type III** Hiba Daghar[1,2], Blake Pyman[3], Alex Parker[2,41]CRCHUM, [2]Université de Montréal, [3]Modelis Inc., [4]Neurosciences, CRCHUM

Rare congenital diseases are in urgent need of therapies. A simple animal such as the nematode *Caenorhabditis elegans (C. elegans)* may provide insights into the investigation of these diseases. Because of its highly conserved genome through evolution, it is possible to investigate underlying pathways of several disorders. In this study, we characterize glycogen storage disease type III (GSDIII), also known as Cori disease in patient-specific *C. elegans* mutants.

GSDIII is caused by a mutation in *AGL* gene coding for glycogen debranching enzyme which is involved in the glycogenolysis pathway. Clinically, GSDIII patients have significant liver hyperplasia, hypoglycemia, mental retardation, and myopathy. The *C. elegans* orthologue of the Human *AGL* gene is *agl-1*. Our previous work on whole gene deletion (*agl-1 (deletion))* and point mutation strains (*agl-1 (W1044X), agl-1(S1444R*)) showed significant phenotypes including, most interestingly, motility defects ang glycogen buildup.

Based on our primary observations, we accomplished an unbiased drug screening on motility phenotype after which we accomplished a RNAi screening. Common genetics targets clustered from top hit compounds were knocked down (77 genes). We identified 8 genes that significantly change glycogen buildup in *agl-1(S1444R)* from which *chk-1* was able to rescue several phenotypes, suggesting that checkpoint signalling pathway might be involved in GSDIII physiopathology. An update of our work will be presented.

Keywords: *Caenorhabditis elegans*, rare disease, glycogen storage disease, characterization

1189C **A post-mortem on a glycolytic drug target; When a drug target isn't so wonderful after all?** David Moody[1,2], Collette Britton[1], Tony Page[1], Andrew Plant[2], Tim Geary[2,31]SBOHVM, University of Glasgow, [2]Animol Discovery, [3]Institute of Parasitology, McGill University

Anthelmintic resistance is an ever-increasing problem which, coupled with the slow pace of novel anthelmintic discovery and development, leads to difficulties in managing parasitic infections. DNA-encoded library (DEL) screening provides an opportunity to quickly and cost effectively discover novel anthelmintics by screening billions of small molecules against target proteins. This target-based approach provides an opportunity to bring anthelmintics to market where the mechanism of action is already fully understood. These anthelmintics are further enhanced by having selectivity for the parasite and not harming the host built in right from the first step.

DEL offers the solution to discovering completely novel drug classes to aid in turning the tide of anthelmintic resistance. Except, it requires a viable drug target. The protein must amenable to expression, to get pure protein for the DEL.

This case study takes us through a glycolytic enzyme picked as an 'obvious' drug target in *C. elegans*, used as a stand in for parasitic nematodes targets. Although initially promising this project did not yield the anthelmintics promised through no fault of the DEL or the chemistry itself. How can a project fail at the protein target level.

Despite being highly conserved and single copy across nearly all life, digging deeper into its function in *C. elegans* reveals are more complex story which suggests that it isn't as druggable a target as it appears, with risks of non-essentiality, and duplicate copies in key target nematodes.

1190C **Host-pathogen interaction between *C. elegans* and the oomycete pathogen *Haptoglossa zoospora*** Kenneth Liu, Manish Grover, Michalis Barkoulas Imperial College London

We recently discovered a new natural pathogen of *C. elegans*, the oomycete *Haptoglossa zoospora,* an extremely potent

pathogen capable of eradicating entire populations of *C. elegans*. *H. zoospora* infection starts with specialized infection cells called gun cells, which attach to the cuticle and fire a single-cell sporidium into the host body using a needle-like structure. Afterwards, *H. zoospora* grows inside the host and digests internal tissues whilst leaving the cuticle intact.

Using this system, we are investigating the virulence factors secreted by *H. zoospora* during infection and how *C. elegans* can respond to and defend itself against *H. zoospora*. We found that *C. elegans* can recognise *H. zoospora* in the absence of infection, activating a transcriptional defence response characterized by the expression of multiple *chitinase-like* genes that antagonize oomycete infection. We report that members of the highly expanded C-type lectin-like family, CLEC-26 and CLEC-36, are required for this response and may act as pattern recognition receptors. We show that neuronal *clec-26* and *clec-36* expression drives *H. zoospora* recognition, and more specifically, that *clec-26/36* expression in only the AWA neurons is sufficient to rescue defective *H. zoospora* recognition in *clec-26/36* double mutants.

In addition to oomycete-specific induction of chitinase-like genes upon *H. zoospora* recognition, we report upregulation of a previously uncharacterised serine-protease inhibitor *Y49G5A.1,* with hypodermal overexpression of *Y49G5A.1* increasing survival of animals against *H. zoospora* infection. Furthermore, we found that hypodermal expression of *H. zoospora* serine proteases can kill animals and that this phenotype can be rescued by expression of the *C. elegans* serine protease inhibitor *Y49G5A.1*. These findings increase our understanding of the arms race between *C. elegans* and its natural oomycete pathogens.

1191C **A role for zinc in regulating the response to oomycete infection.** Jonathan Saunders, Manish Grover, Michalis Barkoulas Imperial College London

Oomycetes are fungal-like eukaryotes closely related to brown algae. Oomycetes such as *Mycozytiopsis humicola* and *Haptoglossa zoospora* are natural pathogens of *C. elegans* allowing for the study of oomycete-animal interactions in a well-established animal host. *C. elegans* can detect the presence of oomycete and elicit an immune response known as the Oomycete Recognition Response (ORR). A hallmark of this response is the induction of *chitinase-like* (*chil*) genes in the hypodermis, which are thought to play a role in cuticle remodelling and protection against oomycete infection. The promotor of one of the most highly induced *chil* genes, *chil-27*, has been utilised in our lab to create a *chil-27p::GFP* transcriptional reporter that gives a robust readout of oomycete detection upon treatment with an innocuous pathogen-derived extract.

Through an EMS screen, we found that a missense mutation in a zinc transporter, *zipt-7.2*, causes constitutive activation of the *chil-27p::GFP* reporter and the induction of ORR genes. This zinc transporter is required in the hypodermis to modulate *chil-27* induction acting alongside other hypodermal factors in the response. A suppressor EMS screen identified mutations in other zinc transporters, namely *cdf-1* and *zipt-2.4*, which suppress the constitutive activation of the *chil-27p::GFP* reporter caused by the *zipt-7.2* mutation. Furthermore, *cdf-1* mutants have reduced *chil-27p::GFP* induction while zipt-2.4 mutants have increased *chil-27p::GFP* induction upon oomycete detection. Zinc is an essential trace element acting as a catalytic and structural factor of hundreds of proteins. However, the role of zinc in *C. elegans* innate immune signalling remains unknown. By utilising mutants for these zinc transporters and manipulating the zinc environment, we propose a model where high cytosolic zinc suppresses the immune response and low cytosolic zinc increases the immune response upon oomycete detection. These results suggest that zinc homeostasis is an important factor for modulating the response to oomycete recognition in *C. elegans*.

1192C **Aging-related changes in cell morphology** Nilay Gupta, Anke Kloock, E. Jane Albert Hubbard Cell Biology, NYU Grossman School of Medicine

Cellular morphology is one of the several factors that plays an important role in cell function. From the biconcave shape of red blood cells to the long-branched morphology of neurons, many cells have specialized morphology that facilitates their function. Cells that participate in direct cell-cell contact and contact-dependent intercellular signaling may be especially reliant on their morphology. Age-dependent disruption in cell shape may lead to many pathologies resulting from impaired function. How organismal age impacts cell morphology is not well understood.

To understand how aging affects cell morphology, we are studying the adult hermaphrodite distal tip cell (DTC). The adult DTC has a complex morphology. It produces membrane-bound ligands for the germline-expressed Notch receptor, GLP-1, the activity of which specifies germline stem cell fate. Previous work indicated that the germline stem/progenitor pool becomes depleted with age [Qin and Hubbard (2015) *Nat Commun*; Kocsisova *et al*. (2019) *Development*], and *daf-2* (IGF-insulin receptor-like) mutants resist this depletion in a manner dependent on the DAF-16 (FOXO) transcription factor and on germline flux. This work showed that *daf-16* activity is required germ cell non-autonomously to control the adult progenitor pool with age. We found that it acts in the proximal somatic gonad, a somatic location that is anatomically distinct from its role in regulating lifespan downstream of *daf-2* [Qin and Hubbard (2015) *Nat Commun*].

Here, we use live imaging of the DTC to show that its morphology radically changes with age, and we assess the role of *daf-*

2 in age-related changes in DTC morphology. We define three distinct parameters that change with age. We find that they are, respectively: independent of *daf-2*, sensitive to *daf-2* but not to *daf-16*, and sensitive to both *daf-2* and to *daf-16*. Further, our results suggest that *daf-16* regulates DTC morphology non-autonomously, acting from the muscle, a tissue requirement that is distinct from *daf-16* influence on the aging germline stem/progenitor pool and distinct from its influence on longevity. Our results offer a new model for the effects of aging on cell morphology and underscore the necessity to understand the regulation of age-dependent changes in cell morphology at the level of the whole organism.

1193C **Space Microgravity Alters Immune Function and Increases Infection of a Gut-Commensal in *C. elegans*** Alfredo V. Alcantara[1], Eunha Chang[1], Ban-seok Kim[1], Toko Hashizume[2], Akira Higashibata[3], Atsushi Higashitani[4], Nathaniel J. Szewczyk[5], Timothy Etheridge[6], Jin I. Lee[11]Division of Biological Science and Technology, Yonsei University Mirae Campus, [2]Advanced Engineering Services, [3]Japan Aerospace Exploration Agency, [4]Tohoku University, [5]Ohio University, [6]University of Exeter

Deep-space explorations will expose many astronauts to the extensive health-risks of microgravity. Studies on animals and humans have reported a decline in immune functions and reactivated latent-virus infection in space (Sonnenfeld and Shearer, 2002). However, studies on space immunity at the genetic level is still limited, and thus, there is a need for an infection model in space. *Caenorhabditis elegans* is a pioneer model organism that has been successfully cultivated in spaceflight during several missions. Gene expression studies in space have shown that the TGFβ pathway ligand, *dbl-1* gene is downregulated aboard the International Space Station (ISS) (Harada et al., 2016), whereas the p38 MAPK ortholog, *pmk-1*, increases in expression in ground-based simulated microgravity experiments (Li, Wang, & Wang, 2018). To determine whether space microgravity affects immune function, we have dispatched *C. elegans* aboard the ISS in microgravity fed with the commensal bacterium *Enterobacter cloacae* tagged with tdTomato*. E. cloacae* is readily consumed and allows normal growth in N2, but will colonize the intestine of immunosuppressed *dbl-1* mutants (Berg et al., 2019). Results from our space-flown worms have shown a decreased resistance to *E. cloacae* gut colonization compared with Earth gravity control worms suggesting a decline in immune function in space. We observed similar results using 3D clinostat simulated microgravity in the lab. *pmk-1* mutants, however, display increased gut colonization in both space and lab microgravity. Finally, by RNAseq analysis, we have identified downstream effector genes of *pmk-1* that inhibit *E. cloacae* infection in microgravity. Collectively, these results demonstrate changes in immune gene signals that are important to regulate gut-microbe and resist infection in space.

1194C **Investigating the Role of Alternative Splicing in Reproductive Aging** Margaret Champion, Francis Amrit, Arjumand Ghazi University of Pittsburgh

Alternative splicing is a process of mRNA editing that allows for both post-transcriptional regulation and the generation of diverse functional proteins from a single gene. Global dysregulation of alternative splicing is a signature of age, and changes in the splicing patterns of specific genes have been linked to age-related decline. However, the extent to which these changes are casual and not merely indicative of the aging process is not fully understood. Here we investigate the role of alternative splicing in age-related reproductive decline. Our lab has recently shown that the pro-longevity factor TCER-1 is necessary for maintaining reproductive fitness in aging *Caenorhabditis elegans.* We have also demonstrated that TCER-1 regulates alternative splicing processes.

Using *in vivo* and *in silico* approaches, our research aims to establish how age-related changes in splicing patterns and fidelity contribute to reproductive senescence. We identify both specific genes and general processes affected by age-related loss of alternative splicing fidelity, and attempt to determine how TCER-1's role as a pro-longevity, pro-fertility factor relates to its role as a regulator of alternative splicing.

1195C **Elucidating the SREBP/Transketolase lipid homeostasis regulatory circuit** Amandine Rapp[1], Amy K Walker[2], Jennifer L Watts[3], Anders M Naar[1], Veerle Rottiers[11]NST, University of California Berkeley, [2]Program in Molecular Medicine, University of Massachusetts Medical School, [3]Washington State University

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 is currently unclear. We found that SBP-1 and TKT-1 function are closely related, as

the expression of *tkt-1* and a wide range of NADPH-producing enzymes is SBP-1 dependent. We also observed that depletion of NADPH-producing enzymes of the PPP (*gspd-1* and 6PGD (T25B9.9)) strongly upregulates the transcription of NADPH-producing enzymes, including *tkt-1*. This upregulation was found to be mediated by SBP-1, seemingly through increased nuclear localization. This suggests that SBP-1 responds to decreased NADPH levels, allowing a coupling of the induction of NADPH-consuming lipogenic processes to NADPH production. Preliminary data suggests that TKT-1 is also required for the upregulation of its own gene observed upon 6PGD knockdown, and we are currently investigating whether TKT-1 is required for the upregulation of other NADPH-producing enzymes observed upon 6PGD depletion. Further analysis will allow to clarify the role of TKT-1 in lipid homeostasis.

1196C **Metabolic phenotyping of purine nucleotide cycle disorders** Latisha Franklin, Rishika Patil, Wendy Hanna-Rose Pennsylvania State University

According to the NIH Genetic and Rare Diseases (GARD) Information Center, at least 13,000 of the 30 million people who have been diagnosed with a rare disorder have a muscle related or myopathy diagnosis. Due to the range of symptoms relating to myopathy and current testing methods, I propose the reported numbers are an underrepresentation. Metabolic phenotyping draws connections between a patient's physiological and biochemical state aiding in disorder diagnosis and treatment. Inborn errors in purine metabolism, specifically the purine nucleotide cycle (PNC), can result in myopathies such as Distal Myopathy 5 (MPD5), Myoadenylate Deaminase Deficiency (MMDD), and Adenylosuccinate Lyase Deficiency (ASLD). Purine metabolism is important for purine nucleotide synthesis as well as maintaining energy metabolism. I sought to characterize muscle dysfunction and the response of energy metabolism when a PNC enzyme has reduced function. In this work, I use the *Caenorhabditis elegans* model to characterize and study muscle dysfunction associated with PNC disorders. I hypothesized knockdown of each of the enzymes—*adss-1, adsl-1,* and *ampd-1*—would result in suboptimal muscle function and disruptions in steady-state energy metabolism. I use WormLab software to measure parameters of crawling and swimming motility and Liquid Chromatography paired with Mass Spectrometry (LCMS) to gain insight about steady-state metabolic changes. Knockdown of *adss-1* resulted in slower crawling and swimming movement and incoordination. Further, metabolic analysis of *adss-1*(RNAi) showed fluctuations in purine metabolism as well as the TCA cycle. Knockdown of *ampd-1* resulted in more subtle changes, specifically to coordination when measured for a short bout of movement, and energy metabolism remained relatively stable. Subjecting these animals to longer bouts of movement resulted in Swimming Induced Paralysis. Knockdown of *adsl-1* resulted in slower swimming movement and incoordination while crawling and swimming. In conclusion, I have established a *C. elegans* muscle dysfunction and metabolic model of MPD5 and MMDD caused by mutations in *ADSSL1* and *AMPD1*, respectively. My studies have provided a physical and biochemical profile of MPD5, MMDD, and ASLD myopathies and gives insight to potential targets for further therapeutic development.

1197C **An intestinally expressed acetylcholine receptor mediates systemic mitochondrial stress responses** Rebecca J Cornell, Wei Cao, Ava Handley, Roger Pocock Anatomy and Developmental Biology, Monash University

Maintaining mitochondrial function across 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 remain largely unknown. In this study, we aimed to better understand how the nervous system controls distal mitochondrial function and health.

In a screen of neurotransmitter mutants, we found that loss of neuronal gamma-aminobutyric acid (GABA) induces mitochondrial stress responses in intestinal cells. We show that GABAergic motor neurons control intestinal mitochondrial stress responses – including the unfolded protein response and mitochondrial dynamics – by regulating acetylcholine signalling through the metabotropic $GABA_B$ receptor complex. When the inhibitory GABA signal is lost, cholinergic neurons are overactive, leading to increased acetylcholine release. In support of this, increased systemic acetylcholine in the *ace1; ace2* compound mutant – which is defective in degrading and inactivating acetylcholine – also induces a mitochondrial stress response and mitochondrial fragmentation in the intestine. Further, we identified an intestinally expressed nicotinic acetylcholine receptor that is essential for GABA/acetylcholine signalling to regulate the mitochondrial stress response. Thus, our data reveal a pathway where GABA/acetylcholine signalling converges on a specific acetylcholine receptor to mediate the mitochondrial unfolded protein response and mitochondrial dynamics in distal tissues.

1198C **Elucidating the Connection between mTORC2 and Metabolism** Elise N Garner[1], Chester J. J. Wrobel[2], Frank C. Schroeder[21]Molecular Biology and Genetics, Cornell University / Boyce Thompson Institute, [2]Chemistry and Chemical Biology, Cornell University / Boyce Thompson Institute

The bacteria-derived metabolite rapamycin inhibits mTOR, an essential protein kinase that is highly conserved in eukaryotes[1,2].

mTOR exists in two complexes, mTORC1 and mTORC2, that share the proteins mTOR and mLST8[2]. Important proteins unique to each complex are RAPTOR (in mTORC1) as well as RICTOR and mSIN1 (in mTORC2)[2]. Rapamycin has enabled detailed studies of mTORC1-mediated metabolic regulation that have revealed mTOR as a "master regulator" of metabolism[1,3]. mTORC1 is activated by nutrients indicative of favorable growth conditions such as the essential amino acid leucine[2]. Active mTORC1 halts autophagy, a catabolic starvation response, and promotes the biosynthesis of lipids, nucleotides, and proteins to foster cellular growth and maintain metabolic homeostasis[2]. Although mTORC2 is essential to mTOR function, mTORC2-mediated metabolic regulation is not well understood because of its rapamycin-insensitivity[3]. *Caenorhabditis elegans* is an excellent model for investigating mTORC2 due to the high conservation of mTOR signaling and central metabolism[4]. Furthermore, *C. elegans* mutants *rict-1* and *sinh-1*, orthologs of RICTOR and mSIN1, respectively, are nonlethal[4]. Using mass spectrometry-based comparative metabolomics, I have identified three classes of previously unreported metabolites that are upregulated in loss-of-function mTORC2 mutants compared to wild type (N2): modular glucosides (MOGLs) and carnitine derivatives that incorporate the isoleucine catabolite α-methylcrotonate, as well as *N*-acylethanolamides. These results suggest that mTORC2 may interact with branched-chain amino acid (BCAA)—particularly isoleucine—catabolism, and lipid metabolism. Ongoing work focuses on investigating changes in expression of genes involved in BCAA catabolism and exploring the physiological roles of the newly identified mTORC2-dependent metabolites.

1. Li *et al. Cell Metabol.* (2014)
2. Liu *et al. Nat. Rev. Mol. Cell Biol.* (2020)
3. Szwed *et al. Physiol. Rev.* (2021)
4. Blackwell *et al. Genetics* (2019)

1199C **Exploring the effects of nanoplastics with different surface modifications on *Caenorhabditis elegans*** Ayoung Jeong
Hallym University

Concerns have recently been raised about the impact of microplastics and nanoplastics on animal health. Nanoplastics (size < 100 nm) are small enough to potentially cross the blood-brain barrier and enter the brain. However, the precise mechanisms underlying their *in vivo* toxicity remain unclear. Here, we investigated the physiological effects of nano-polystyrene beads using *Caenorhabditis elegans* as a model system. We discovered 20 nm fluorescent polystyrene beads in its digestive canal after ingesting them, which is a common route for humans to be exposed to nanoplastics. We then investigated the effect of nano-polystyrene beads with different surface functional groups (pristine, carboxyl-, or primary amine group) on animal growth and intestinal damage in *C. elegans*. Exposure to high concentrations of 25 nm pristine nano-polystyrene beads resulted in reduced animal growth, while prolonged exposure to low concentrations of 25 nm amino-modified nano-polystyrene beads resulted in increased gut leakage and mitochondrial fragmentation. These findings imply that nano-polystyrene beads may worsen diseases involving intestinal function and increase oxidative stress. More research is being conducted to investigate degenerative diseases that may be accelerated by chemically modified nano-polystyrene beads.

1200C **The role of *Caenorhabditis elegans huntingtin* in stress response** Christine Chung[1], Hanee Lee[1], Roy Jung[2,3], Ihnsik Seong[2,3], Junho Lee[11]School of Biological Sciences, Seoul National University, [2]Center of Human Genetic Research, Massachusetts General Hospital, [3]Department of Neurology, Harvard Medical School

Huntingtin (HTT) is a casual gene of Huntington's disease (HD), a genetic neurodegenerative disorder. HD is both gain of function of the mutant huntingtin protein and loss of function of the wild-type huntingtin protein. HD patients have abnormal CAG trinucleotide repeat expansion mutation in exon 1 of HTT gene causing neurodegeneration. HTT gene is highly conserved from yeast to human and the gene is essential for maintaining life. According to recent studies wild-type huntingtin protein is involved in a rapid cell stress response. Moreover, the lowered wild-type huntingtin level increases vulnerability to cellular stress. *Caenorhabditis elegans* has putative *huntingtin* ortholog that has no reported known function. *C. elegans huntingtin* homozygous null mutants are viable and this makes *Caenorhabditis elegans* a strong research model to study the normal function of *huntingtin*. The sequence alignment analysis shows that *huntingtin* in *Caenorhabditis elegans* and HTT in human have similar sequences near the C termini. *Caenorhabditis elegans huntingtin* is expressed in multiple tissues and especially enriched in neurons and glial cells. We observed that *huntingtin* in *Caenorhabditis elegans* has roles in stress response pathway. *Caenorhabditis elegans* with deletion mutation in *huntingtin* gene show increased mortality to pathogen infection and to thermal stress, and the expression of wild-type *huntingtin* rescues the *huntingtin* mutant phenotype. In addition, we observed that *huntingtin* in *Caenorhabditis elegans* has prosurvivng role through ERK pathway. Our research evaluating the function of *Caenorhabditis elegans huntingtin* in stress response will help to find the unknown molecular action mechanism of human HTT and to have advances in understanding the pathology of HD.

1201C **Identifying mechanisms underlying intestinal control of neuronal functions** Chung-Chih Liu[1], Nicolas Seban[2], Aayushi Shah[1], Supriya Srinivasan[11]Neuroscience, The Scripps Research Institute, [2]University of California, Davis

The communication between the intestine and neurons is critical to regulating metabolic homeostasis in animals. Our previous work identified a conserved FLP-7/NPR-22 neuroendocrine axis, which underlies the serotonergic control of body fat loss in *C. elegans*. ASI chemosensory neurons can secrete FLP-7 neuropeptide to act in the intestine via the NPR-22 receptor to derepress the expression of ATGL-1 to drive fat loss. Our previous studies also found that the state of intestinal fat reserves is relayed to oxygen-sensing URX neurons and modulates their resting state and evoked responses. Thus, we hypothesized that an intestine-to-neuron signal should exist to relay the metabolic state of the intestine to the nervous system. Although many gut hormones were discovered in the intestine-to-neuron axis, how they modulate neuronal functions and the molecular mechanisms of releasing these regulatory peptides from the intestine are largely unknown.

To search for potential intestine-to-neuron signals that modulate FLP-7 secretion from ASI neurons, we conducted an intestine-specific RNAi screen to target all secreted neuropeptides in the genome and used FLP-7 secretion level as a readout. We identified an insulin superfamily gene called *ins-7*, which negatively regulates FLP-7 secretion. *ins-7* null mutants show increased FLP-7 secretion, elevated energy expenditure, and decreased body fat stores. Further, we found that the phenotypes of *ins-7* null mutants are dependent on *flp-7*. Ongoing experiments aim to decode the effects of this intestinal peptide on ASI neurons that control body fat homeostasis and understand the regulatory mechanisms underlying the release of INS-7 from the intestine. We expect our studies will identify novel molecular mechanisms by which sensory or metabolic information from the intestine modulates the functions of the nervous system.

1202C **An ancient thyrostimulin-like neuroendocrine system regulates growth and intestinal function in *C. elegans*** Majdulin N Istiban, Signe Kenis, Sara Van Damme, Elke Vandewyer, Jan Watteyne, Liliane Schoofs, Isabel Beets Biology, KU Leuven

Animal growth is supported by energy homeostasis, which is tightly regulated by neuroendocrine factors. The glycoprotein hormone thyrostimulin was recently discovered as a novel regulator of growth, which activates the thyroid stimulating hormone receptor (TSHR) in mammals. Orthologs of the thyrostimulin subunits, GPA2 and GPB5, are highly conserved in both vertebrates and invertebrates. However, thyrostimulin's functions remain largely unexplored and its mode of action in growth regulation has not been studied in any animal. To investigate this, we have identified and functionally characterized a thyrostimulin-related signaling system in *C. elegans*. The *C. elegans* genome encodes two orthologs of the thyrostimulin GPA2 and GPB5 subunits, which we called *gpla-1* and *gplb-1*. In addition, *C. elegans* has only one glycoprotein hormone receptor ortholog, called FSHR-1, which is still an orphan receptor. We found that GPLA-1 and GPLB-1 activate FSHR-1 *in vitro*, resulting in increased cAMP signaling, and are cognate ligands of this receptor *in vivo*. Like in mammals, thyrostimulin-like signaling also promotes growth in *C. elegans*. Thyrostimulin's subunits are primarily expressed in the pharyngeal and defecation motor circuit, suggesting a role in the regulation of feeding and intestinal function. We discovered that GPLA-1/GPLB-1 signaling promotes growth by activating FSHR-1 in glial cells and the intestine. Mutants defective in thyrostimulin-like signaling display bloating of the intestinal lumen and have defects in defecation behavior. In addition, we found that thyrostimulin and neuropeptides related to mammalian thyrotropin-releasing hormone (TRH) act in the same growth-regulating pathway. Our findings suggest that thyrostimulin is an ancient enteric neuroendocrine factor that regulates intestinal function and may ancestrally have been involved in the control of postembryonic growth.

1203C **Folate receptor in health and aging** Bideep Shrestha[1], Olli Matilainen[21]University of Helsinki, [2]Organismal and Evolutionary Biology Research Programme, University of Helsinki

Folate, also known as vitamin B9, is a co-factor in one-carbon metabolism. It is important factor in epigenetic maintenance, redox defense, and biosynthesis of different nucleic- and amino acids. Hence, this vitamin is essential for development and health. *C. elegans* has two identified folate transporters, FOLT-1 (ortholog of reduced folate carrier, the major folate transporter in *C. elegans*) and FOLR-1 (ortholog of folate receptor). Whereas FOLT-1, which is expressed ubiquitously, is required for normal health- and lifespan, the role of FOLR-1 in animal physiology is still largely unknown. We found that FOLR-1 is expressed only in vulval cells, thus mimicking the restricted expression profile of human folate receptors. Interestingly, we discovered that knockdown of LIN-53 leads to ectopic FOLR-1 expression, demonstrating that this histone-binding protein is required to maintain FOLR-1 tissue specificity. Interestingly, depletion of FOLR-1 does not affect health- or lifespan, thus supporting earlier finding that FOLR-1 is not essential for the uptake of folates as vitamins. It is well-established that folate receptor shows strongly induced expression in multiple cancers, and therefore, we asked whether *folr-1* overexpression (OE) affects organism's physiology. Strikingly, we found that *folr-1* OE impairs health and shortens lifespan. When elucidating the mechanisms behind these phenotypes, we discovered that *folr-1* OE reduces the level of ribosomal subunit in body-wall muscle cells. Together, our data reveal that the expression of FOLR-1 is tightly controlled, and that increased *folr-1* expression deteriorates health. Furthermore, we show that *folr-1* overexpression reduces the level of ribosomal component in body-wall muscle cells, suggesting that it affects protein

synthesis in distal tissues. Regarding the clinical relevance of our data, large proportion of cancer patients suffer from cachexia, which manifests in loss of skeletal muscles due to reduced protein synthesis and increased degradation. Therefore, our findings highlight folate receptor, which is typically overexpressed in cancer, as a possible factor contributing to cancer-associated cachexia.

1204C **Peroxisomal biogenesis as a potential target for polyglutamine disorders.** Konstantinos Kounakis, Maria Markaki, Nektarios Tavernarakis Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, IMBB FORTH

Polyglutamine disorders, such as Huntington's disease and certain types of Spinocerebellar Ataxia are the result of abnormal expansion of CAG glutamine codon repeats in important genes. This expansion leads to the synthesis of elongated polyglutamine (polyQ) chains in the affected protein and the formation of aggregates. In patients, this is associated with the gradual development of severe neurodegenerative phenotypes such as motor and cognitive deficits. Studies in patients and animal models have suggested that lipid and oxidative metabolism are connected to the emergence of these symptoms. They have also specifically implicated PPAR-δ and PGC-1α as important factors mediating disease pathogenesis. Taken together, these findings suggest that peroxisomes, with their prominent auxiliary role in lipid metabolism and their central role in ROS regulation could also be key players in polyQ-induced neurodegeneration. To test this hypothesis, we examined the effects of manipulating peroxisomes on the formation of polyQ aggregates in *C. elegans* models of the disease. We demonstrate that the inhibition of peroxisomal biogenesis during development can significantly delay the formation of large polyQ inclusions. We are currently dissecting the cellular and molecular mechanisms underlying these neuroprotective events.

1205C **Crosstalk between lipid metabolism and mitochondrial function in neurodegeneration** Dikaia Tsagkari, Maria Markaki, Nektarios Tavernarakis FORTH-IMBB

Whole-body energy metabolism is regulated by a superfamily of nuclear hormone receptors, named peroxisome proliferator-activated receptors (PPARs) that act as lipid sensors. Alterations in cellular energy metabolism are pathophysiological features of many neurodegenerative disorders. In addition, activation of PPARs has been reported to protect against neurodegeneration. However, the molecular mechanisms that mediate neuroprotection remain elusive. We hypothesized that PPARs link alterations in mitochondrial function to lipid metabolism, under both physiological and disease contexts. To test this hypothesis, we functionally characterized the role of *C. elegans* PPAR orthologues in lipid metabolism and mitochondrial function. Our results suggest that PPARs directly regulate fat accumulation, formation of lipid droplets, as well as mitochondrial function and dynamics. In addition, we find that PPARs exert neuroprotective effects in two different models of neurodegenerative diseases. We are currently investigating how modulation of PPAR activity coordinates lipid metabolism and mitochondrial dynamics, to ultimately influence neuronal integrity and function.

1206C **A genome-wide screen for tumor regulating genes** Jiangying Wei[1,1], Jingyi Chen[2], Bing Han[1,21]The Shanghai Key Laboratory of Medical Epigenetics, International Co-laboratory of Medical Epigenetics and Metabolism, Ministry of Science and Technology, Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China, [2]Institute of Pediatrics, Children's Hospital of Fudan University, Shanghai 201102, China

Various malignant tumors have become major health threats and causes of medical burdens nowadays. The relationships between tumor onset and individual genes are complex, therefore, the strategy of tumor suppression via modified gene expression is theoretically sound. However, to date, no systemic studies on these genes have been conducted to reveal their identities or mechanisms. Here, we used a model organism, the nematode *Caenorhabditis elegans*, to conduct a genome-wide screen in order to identify all genetic modifications effective in antagonizing tumors. The merits of *C. elegans* including short life cycle, high reproductive ratio, as well as molecular similarities to humans make them especially suitable for this high-throughput purpose. Briefly, we inactivated each individual gene using RNA interference and then determined its influences on progression of two distinct germline tumors, exhibited by a gain-of-function mutant of Notch receptor *glp-1(ar202)* and a Quaking inactivation mutant *gld-1(q485)*, respectively. Our screen has led to identification of dozens of genes whose downregulation remarkably alleviated both tumor pathologies, either by prolonging survival or by allowing normal germ cell development to produce viable offspring. The most prominent screening hit with clear human homologues is the *suco-1* gene, known to participate in endocytosis and cell secretion. Tissue-specific RNAi revealed that the tumor suppression of *suco-1* RNAi effects mainly through intestine and muscle, thus representing a cell non-autonomous mode of action. This study is insightful in discovering novel tumor regulating genes in an exhausting manner, with the *suco-1* gene as a typical case in point. Practically, our screen will also unravel many potential targets for curing tumors.

1207C **Regulation of fat metabolism by minimally altered gut microbiota** Yunhan Wang[1], Minbiao Ji[2], Bing Han[31]Institutes of Biomedical Sciences Fudan University, [2]STATE KEY LABORATORY of SURFACE PHYSICS Fudan University, [3]Institutes of Biomedical Sciences Fudan University, Institutes of Children's Hospital Fudan University

Fat metabolism disorders, represented by obesity and tightly linked to multiple chronic pathologies such as diabetes and cancer, constitute a prevalent medical challenge throughout the world. Recent studies have implicated that gut microbiota plays an essential role in regulating fat storage and expenditure. However, no responsible microbial factor has been identified so far, mostly due to the constant changing state and the compositional complexity of typical gut flora. We utilized the model organism *Caenorhabditis elegans* to solve this puzzle, taking advantages of their clearly defined microbiota. By deleting each single bacterial gene while controlling other backgrounds, we developed a 'probiotic' screening system to identify fat-regulating microbial factors. Stimulated Raman scattering microscopy was employed to directly visualize and quantify the fat content in nematode intestines, greatly facilitating the screening process.

Among the screening hits, the most prominent are bacterial mutants #168 and #275, which on average decreases the host fat storage by 32% and increases it by 41%, respectively. This alteration is highly conserved in both sexes of a distant relative of the hermaphroditic *C. elegans*, namely *C. remanei*. Ruling out species- and gender-specific mechanisms optimizes their future translational values. Furthermore, we found that upon #168 colonization, a host transmembrane receptor Str-7 and a lysosomal lipase Lipl-4 are remarkably upregulated via qRT-PCR. Meanwhile, knocking down of *str-7* by RNAi completely abolishes the fat reduction modulated by #168, confirming its role as a central regulator of this bacteria induced response. As for the effect of #275, we similarly demonstrated that a host peroxisomal fatty acid CoA synthetase Acs-7 is both sufficient and necessary for this microbiome regulated fat accumulation. Collectively, our results highlighted the host lysosomes and peroxisomes as the sites for host-microbiota interactions, and these two organelles are potential targets for the management of fat metabolism.

As the initial step towards the clinical application of our findings, we provided these two bacterial mutants to C57BL/6J mice using gastric gavage. By far, the conservation test results obtained from several individuals were positive, making our work highly promising in controlling obesity. The concept of purposely designing microbiota to cure metabolic diseases becomes more feasible than ever.

1208C **Age-related phenotypes caused by an Alzheimer disease-related presenilin-1 protein splice variant in *Caenorhabditis elegans*** Carla Almendariz-Palacios[1], Cheng-Wei Wu[2], Darrell D Mousseau[1], Christopher Eskiw[11]Department of Food and Bioproduct Sciences, University of Saskatchewan, [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan

As we age, our brain undergoes several changes at the cellular and molecular levels. These changes, including processes associated with the loss of proteostasis, lead to the aggregation of misfolded proteins and to the disruption of the nuclear membrane. Age-related disruption of these mechanisms is strongly associated with diseases, such as Alzheimer's disease (AD). On the other hand, AD brain burden is commonly related with the production of b-amyloid due to the incorrect cleavage of the amyloid precursor protein (APP). APP incorrect cleavage has been linked with dysfunction of the presenilin-1 (PS-1) protein. Alterations in the *PSEN-1* gene sequence have been related with nuclear membrane disruption and autophagy impairment. A screen of mRNA transcripts in our laboratory identified a splicing variant of PS-1 (PS-1 (SV)) that is highly expressed in AD brain samples; however, its role during AD and aging is unknown.

We hypothesize that PS-1 (SV) expression can lead to age-related phenotypes such as the nuclear membrane disruption and autophagy (cellular self-eating process) impairment. To test our hypothesis, we utilize the *C. elegans*model, widely used to study aging and neurodegeneration. The *sel-12(ar131)* strain, which carries a loss-of-function SEL-12, worm ortholog of PS-1, exhibits egg-laying defects, reduced lifespan, nuclear membrane disruption and loss of proteostasis, due to the inhibition of autophagy triggered by the hyperactivation of the target of rapamycin complex 1 (TORC1), an important nutrient sensing regulator.

Expression of human WT *PS-1* in the *sel-12* mutant, rescues egg-layings defect and re-establishes normal nuclear membrane, while the *PS-1(SV)* cannot. Furthermore, PS-1 (SV) reduces the lifespan of the worm and induces the activation of TORC1. These data indicate that the PS-1(SV) may lead to age-related phenotypes, which might underlie its influence on the development of AD. Future studies will be aimed at alleviating the negative phenotypes of PS-1(SV) expression.

1209C **Effects of microgravity and below-background radiation in *C. elegans*-Orsay virus dynamics** Ana Villena Giménez[1], Victoria G. Castiglioni[2], Rubén González[3], Santiago F Elena[1,41]Instituto de Biología Integrativa de Sistemas (I2SysBio), CSIC-Universitat de València, 46980 Valencia, Spain, [2]Instituto de Biología Integrativa de Sistemas (I2SysBio), CSIC-Universitat de València, 46980 Valencia, Spain., [3]Institut de Biologie de l'École Normale Supérieure, Paris, France, [4]Santa Fe Institute, Santa Fe, NM 87501, USA

In the near future, space travel will be accessible for common citizens, not only astronauts, and space missions will extend its duration to satisfy space exploration goals. Spatial conditions comprise stresses that organisms have not encountered during its evolution, being microgravity and radiation the most dramatic. However, little is known about how spatial conditions impact viral infections and host antiviral defences. Environment plays a crucial role in host-pathogen interactions, leading to variations in infection's dynamics.

C. elegans has already been employed in space research due to its many advantages as a model organism and the pathosystem *C. elegans*-Orsay virus (OrV) is an emerging model to study host-pathogen interactions. OrV is the only known natural virus of *C. elegans* that infects intestinal cells, producing mild intestinal symptoms and little effects on host's fitness.

In this study, we examined the progression of viral load from L1 stage to L4 in populations of *C. elegans* under microgravity and below-background radiation conditions. Microgravity (0.001·g) is simulated using a random position machine (RPM), a device that cancels out gravity vector by rotating in the three axes of space. Experiments in below-background radiation conditions were performed in an ca. 800 m deep underground laboratory that blocks the impact of muons' radiation, the most abundant cosmic radiation at the surface. To increase our understanding of the interplay of microgravity and below-background radiation, we performed the experiments merging both stresses.

We reported a change in viral load profile when infection progresses in the three stressful environments: microgravity, below-background radiation and both together. Likewise, we show the effect of these stresses in the viability and fecundity of eggs. This suggest that viral infection can be modulated by spatial conditions, a critical finding for spatial missions.

1210C **Effects on the *C. elegans* germline in response to infection by *Pseudomonas aeruginosa*** Daniel P Bollen[1,2,3], Kirthi C Reddy[2,4], Dennis H Kim[1,3], Monica P Colaiacovo[51]Infectious Diseases, Boston Children's Hospital, [2]Biology, MIT, [3]Pediatrics, Harvard Medical School, [4]Molecular Neurobiology Laboratory, Salk Institute, [5]Genetics, Harvard Medical School

Infection of *C. elegans* with pathogenic bacteria may affect host evolutionary fitness not only through lethality to the host organism but also with insult to progeny production. Here, we show that infection of *C. elegans* hermaphrodites with the gram-negative pathogen *Pseudomonas aeruginosa* leads to a dramatic reduction in brood size. We observe a concomitant reduction in the number of germ cell nuclei and in the size of the gonad arms. We find that at least two processes are induced that decrease the number of germ cell nuclei. First, we observe that infection with *P. aeruginosa* leads to the induction of programmed germ cell death, as has been previously described for infection with *Salmonella enterica*. Second, we observe that exposure to *P. aeruginosa* induces mitotic quiescence in the proliferative zone of the *C. elegans* germline. Importantly, these processes appear to be reversible; when animals are removed from the pathogenic lawn milieu, germ cell death is abated, germ cell nuclei numbers increase, and brood sizes recover. The observed germline changes may represent an adaptive response to improve survival of progeny in the presence of pathogen or may facilitate resource allocation meant to ensure host survival during pathogen infection.

1211C **Regulation of poly-glutamine protein aggregation and proteostasis by the ubiquitin-like protein UFM-1** Samantha Moores, Betty Ortiz Bido, Peter JuoTufts University Graduate School of Biomedical Sciences

Ubiquitin-Fold Modifier-1 (UFM-1) is a ubiquitin-like molecule (UBL) that has been implicated in protein quality control, however its physiological functions are still poorly understood. Conjugation of UFM-1 (Ufmylation) to substrates is mediated by a series of enzymes including an E1 activating enzyme (*uba-5*), an E2 conjugating enzyme *(ubc-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. We found that loss of function mutations in *uba-5*, *ubc-1* and *ufl-1* leads 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. The UFM-1 pathway has also been shown to regulate the ER Unfolded Protein Response (UPR^{ER}) (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 and that *ufl-1* mutants have shortened lifespan, likely through the pathway's involvement in the UPR^{ER}. Future work will focus on further investigating the relationship between the UFM-1 pathway and the UPR^{ER} and the mechanism by which UFM-1 pathway function at the ER regulates cytosolic aggregation of polyQ proteins.

1212C **Understanding the role of *crh-1* circRNAs in aging and age-related diseases** Hussam Z. Alshareef, Aja McDonagh, Emmanuel Adeyemi, Alexander M. van der Linden Biology, University of Nevada, Reno

Circular RNAs (circRNAs) are largely non-coding RNAs produced via back-splicing. We previously reported that hundreds of circRNAs accumulate during aging in *C. elegans* (Cortés-López *et al*. 2018). In *C. elegans*, one of the most abundant circRNAs that accumulated during aging is circ-*crh-1*, which is a circRNA derived from the *crh-1 gene,* an ortholog of mammalian CREB. Using CRISPR-Cas9, we abolished circ-*crh-1* expression in worms without disrupting the linearly-spliced *crh-1* mRNA. We reported that loss of circ-*crh-1* significantly extended the mean lifespan of *C. elegans* (Knupp *et al.* 2022). Our preliminary results also shows

that loss of circ-*crh-1* improves Ab-induced paralysis in a transgenic *C. elegans* Alzheimer strain that carries the full-length of human amyloid-beta peptide (Ab) in the muscles. Together, this suggests an important role of circ-*crh-1* in aging and age-related disease. To further understand the role of circ-*crh-1* in aging and age-related diseases, we tested additional age-related phenotypes such as thermotolerance, oxidative-stress, and stress-induced sleep (SIS). We found that circ-*crh-1* mutants are sensitive to heat stress and paraquat, suggesting that lifespan extension may led to an increase in frailty. No significant change was observed in SIS in circ-*crh-1* mutants compared to wild-type. Together, these results suggest that circ-*crh-1* plays a critical role in aging and age-related diseases. Future experiments will determine the mechanisms underline these age-related phenotypes.

1213C **Identification of a novel family of benzimidazole complex I inhibitors as potential anthelmintics** Taylor Davie[1], Xènia Serrat[1], Jamie Snider[1], Igor Štagljar[1], Hiroyuki Hirano[2], Nobumoto Watanabe[2], Hiroyuki Osada[2,3], Andrew Fraser[11]Molecular Genetics, The Donnelly Centre, University of Toronto, [2]RIKEN Center for Sustainable Resource Science, [3]Pharmaceutical Sciences, University of Shizuoka

Soil-transmitted helminths (STHs) are major human pathogens infecting over a billion people worldwide. While preventative chemotherapy remains the primary means of controlling parasitic nematode infections, the emergence of anthelmintic resistance has threatened the efficacy of existing drugs. It is thus imperative that new anthelmintics with novel modes of action be developed. Here we focus on identifying new compounds capable of interfering with the unique anaerobic metabolism used by STHs while inhabiting the hypoxic conditions of the host gut. This form of metabolism is known as the NADH-fumarate reductase system or fumarate respiration, and while it is essential to parasite survival, it is not used in humans or any other STH hosts. We previously showed that *C. elegans* is also capable of performing this anaerobic metabolism and established a high-throughput assay in which to study its use. We screened a library of 480 natural products for compounds that kill *C. elegans* under conditions when they rely on fumarate respiration. We identified several new classes of compounds including a novel family of mitochondrial NADH:Ubiquinone oxidoreductase (complex I) inhibitors. These Complex I inhibitors are based on a benzimidazole core but unlike commercial benzimidazole anthelmintics, they do not target microtubules. We screened over 1,200 related benzimidazoles and identified potent analogs with increased activity towards *C. elegans* compared to human cells. As complex I plays an essential metabolic role in both the aerobic and anaerobic life stages of STHs, we suggest these new benzimidazole species-selective complex I inhibitors may have anthelmintic potential.

1214C **Amphid Sensory Neurons of *Caenorhabditis elegans* Orchestrate Host Survival during Infection with Broad Classes of Pathogens** Siddharth Ravi Venkatesh[1], Anjali Gupta[21]Developmental Biology & Genetics, Indian Institute of Science, [2]Center for BioSystems Science and Engineering, Indian Institute of Science

The survival of a host during infection relies on its ability to rapidly sense the invading pathogen and mount an appropriate response. The bacterivorous nematode *Caenorhabditis elegans* lacks the majority of traditional pattern recognition mechanisms that senses pathogens. We hypothesized that the 12 pairs of amphid sensory neurons in the head of worms provide sensing capability and thus mediate host survival during infection. We tested animals lacking functional amphid neurons with three major classes of pathogens, namely – a Gram-negative bacterium *Pseudomonas aeruginosa*, a Gram-positive bacterium *Enterococcus faecalis* and a pathogenic yeast *Cryptococcus neoformans*. Using individual neuronal ablation lines or mutants lacking function in specific neurons, we demonstrate that 5 of the 12 amphid neuron pairs broadly suppress host survival and affect colonization by all pathogens, while 3 amphid neuron pairs were indispensable for survival on all pathogens. The other amphid neurons differentially regulate host survival in a pathogen-specific manner. Overall, our study reveals broad and unique roles for amphid neurons in mediating host survival during infections.

Keywords: *Caenorhabditis elegans*, amphid sensory neurons, pathogens, survival

1215C **Investigating the roles of NAD^+ consuming enzymes on reproduction and aging** Abdulkareem A Alshaheeb, Ian Argento, Jaedon Sadler, Shaina Johnson, Cole Caron, Melanie McReynoldsDepartment of Biochemistry and Molecular Biology, The Pennsylvania State University

Nicotinamide adenine dinucleotide (NAD^+) is an essential coenzyme in redox reactions and co-substrate for signaling enzymes. Literature documented NAD^+ levels can decline with age in human, mice, and worms, resulting in metabolic disruptions that contribute to the development of age-related diseases. Two mechanisms proposed for this decline in organismal NAD^+ concentrations, less synthesis or more degradation of the NAD^+ chemical background. However, A recent study has shown that the rate of new NAD^+ synthesis remains unchanged in aged mice, and the decrease in NAD^+ levels that occurs with age is not due to a lack of precursors. In addition, aged mouse tissues with lower NAD^+ concentrations have correspondingly faster fractional turnover, preserving the total flux. Collectively this work suggests increased consumption is the primary driver of the decline in steady-state NAD^+ concentrations that occur with age. Therefore, I hypothesized that NAD^+ metabolic robustness and resiliency are lost with age due to the hyper-activation and competition of NAD^+-consuming enzymes. To investigate the

impact of deficient NAD^+ consuming enzymes on metabolic robustness and resiliency, we used a combination of genetics and mass spectrometry to explore this relationship. Surprisingly, we identified novel reproductive and aging phenotypes associated with the loss of NAD^+ consuming enzymes, including Poly Adp-Ribose (ADP-ribose) Polymerase (parp-1 and parp-2), TANKyrase (tank-1), and Toll and Interleukin 1 Receptor (tir-1). These mutant animals exhibited significant reductions in both fecundity and longevity. Additionally, we performed High-Performance Liquid Chromatography coupled to Mass Spectrometry (HP-LC-MS) to examine the NAD^+ metabolome through various developmental stages in our mutant strains and uncovered significant metabolic disruptions. Finally, we examined the impact of NAD^+ consumer loss on mobility using WormLab and quantified locomotion defects. Taken together, our findings outline a novel connection between hyper-activation of NAD^+ degradation and the triggering of reproductive aging.

1216C **BMP signaling in regulation of *C. elegans* lipid homeostasis and immune response** Katerina Yamamoto, Cathy Savage-Dunn CUNY Queens College & The Graduate Center

Research on innate immunity has focused on mechanisms that decrease pathogen load, including physical barriers and antimicrobial peptides (AMPs). Less is known, however, about how host metabolism may support survival. Bone Morphogenetic Proteins (BMPs) are secreted peptide growth factors in the TGF-β family, well known for their roles in development and differentiation, but emerging as homeostasis modulators. The DBL-1/BMP Pathway in *Caenorhabditis elegans* regulates innate immunity and lipid metabolism. Up until now, these physiologies have appeared separate, however, here we begin to explore their relationship. We find that pathogen exposure elicits a BMP-dependent lipid mobilization response in the host, and hypothesize that BMP regulation of lipid metabolism in *C. elegans* contributes to survival on pathogenic bacteria. To complement our investigation, we conducted a genetic screen to identify novel genes that function downstream of DBL-1 in lipid homeostasis. We analyzed 8224 genomes and isolated 71 candidate suppressors. Our goal is to define the molecular pathways that act downstream of DBL-1 to regulate lipid metabolism and immune response.

1217C **Pathogen apathy resulting from SKN-1 activation: a mark of resilience or foolish behavior?** Brandy Weathers, Nicole Stuhr, Tripti Nair, James Nhan, Sean CurranUniversity of Southern California

Innate immunity is a key driver in promoting survival against various xenobiotic stressors and hostile environments and an appropriate immune response is essential to organismal health across lifespan. *Pseudomonas aeruginosa* (PA14) is a human opportunistic pathogen that is lethal to *C. elegans* with prolonged exposure. *Caenorhabditis elegans* employ multiple defense responses to ensure survival upon exposure to pathogens to defend against the current infection and to avoid continued exposure including activation of the cytoprotective transcription factor SKN-1. Among the physiological consequences of pathogen exposure is the rapid depletion of stored intracellular lipids in the intestine; a phenotype that manifests even before pathogen colonization and intestinal bloating. Although wild type *C. elegans* quickly learn to avoid pathogens, our current work documents a peculiar apathy to pathogen in animals with constitutive activation of SKN-1. This behavior is mediated by tissue specific actions of SKN-1 that initiate cell non-autonomous outcomes for pathogen avoidance, survival, and metabolic adaptation. Remarkably, although animals with SKN-1 activation are initially apathetic to PA14 exposure, they can still learn to avoid PA14 after a short period of training. We document a unique transcriptional signature of animals with SKN-1 activation upon exposure to PA14 and note that the observed apathy behavior is independent of somatic lipid depletion, thus uncoupling these two exceptional physiological responses. Taken together our work reveals new insights into how animals perceive pathogens in the environment and subsequently alter behavior and cellular programs to promote survival.

1218C **Identifying new gut microbial and host genetic factors that promote healthy ageing** Irtiqa Ali, Alejandra Zarate Potes, Jack Martin, Rajal Patel, Tory Higgins, Natalia da Silva Jardim, Alexandre Benedetto Lancaster University

Since the identification of conserved longevity assurance pathways in *C. elegans*, research in biogerontology has discovered numerous new genes that have small or context-dependent effects on lifespan, and more recently, healthspan. At the same time, the importance of the gut microbiota in animal health and ageing has become increasingly apparent. However, defining the precise molecular mechanisms by which microbiotas impact host ageing and longevity has been challenging, yielding few well-defined examples to date.

In this project, we use high-throughput approaches based on the LFASS automated stress survival assay to screen for gut microbes, new host genes, and host gene-gut microbe interactions that promote healthy ageing. So far, we have examined 46 bacterial isolates for their potential to improve health by protecting adult worms against heat or oxidative stress. We did this in wildtype, insulin/IGF1 signalling mutants and kynurenine pathway mutants. In parallel, we are conducting a genome-wide RNAi screen to identify host genes involved in heat resistance. The hits identified from both bacterial screening and host genetic screens will undergo confirmation and be tested in combination in a secondary screen to assess whether there are any additive or synergistic effects.

Furthermore, we are conducting life trait analyses and simple behavioural assays, such as lifespan, movement coordination, and brood-size, to evaluate longevity, fitness, and healthspan as a follow up to the initial screens. We also plan to generate fluorescently tagged versions of the identified potential probiotic bacterial strains to investigate their ability to colonise and persist in the *C.elegans* gut.

The purpose of this poster is to present an overview of the methods employed, the current results obtained, and planned future work.

1219C **WIPI2B/ATG-18 phosphorylation regulates neuronal autophagosome biogenesis *in vivo*** Heather Tsong[1], Andrea Stavoe[21]The University of Texas MD Anderson Cancer Center UTHealth Houston, [2]The University of Texas Health Science Center Houston

Autophagy is a homeostatic mechanism that cells utilize to clear waste. Autophagy is especially important for neurons because they cannot dilute waste through cell division. However, neurons lose their ability to perform autophagy with age, and the misregulation of autophagy has been implicated in many age-related neurodegenerative diseases (NDDs). Previously, we found that we could restore the rate of autophagosome biogenesis in aged primary murine neurons through the overexpression of WIPI2B, a key autophagy component. Importantly, this rescue is contingent upon the phosphorylation state of WIPI2B. These data suggest that WIPI2B phosphorylation regulates its function in autophagosome biogenesis and that increasing dephosphorylated WIPI2B via protein regulators will upregulate neuronal autophagy. To test this hypothesis, we first verified the role of WIPI2B phosphorylation in neuronal autophagy *in vivo* in *Caenorhabditis elegans*. We generated *C. elegans* strains endogenously expressing phospho-mimetic or phospho-dead ATG-18 (the WIPI2B ortholog). We previously showed that autophagy regulates PVD axon outgrowth cell-autonomously. Using PVD axon length as a simple, visual readout of neuronal autophagy, we determined that worms with phospho-mimetic ATG-18 had longer axons, suggesting defective autophagy, while worms with phospho-dead ATG-18 had normal axon lengths, suggesting functional autophagy. Due to the importance of this phospho-site in autophagy, we used a candidate approach to identify the kinase and phosphatase that regulate ATG-18/WIPI2B phosphorylation. Overexpression of constitutively active CDK-1 in PVD yielded longer PVD axons, suggesting that CDK-1 might regulate neuronal autophagy as a kinase that regulates ATG-18 phosphorylation. Conversely, worms with mutations in a PP2A regulatory subunit SUR-6/B55 displayed longer PVD axons, similarly implicating PP2A in the regulation of neuronal autophagy. Epistatic analysis also suggests that PP2A and ATG-18 function in the same pathway to modulate neuronal autophagy and PVD axon outgrowth. Since autophagy misregulation has been implicated in NDDs, a better understanding of how neurons utilize and regulate autophagy will uncover novel therapeutic targets for treating these NDDs.

1220C **Screening natural products against *Caenorhabditis elegans* to find novel anthelmintic compounds** Sommer Chou, Lesley T MacNeil, Gerard D WrightMcMaster University

Parasitic worm infections affect over a quarter of the human population, reducing quality of life and impacting global health. Despite the severity and frequency of helminthiases, few treatment options are available and rising levels of resistance to existing anthelmintics necessitates a more active drug discovery strategy. In the past, natural products have proven to be a rich source of clinically relevant therapeutics and can be further mined for anthelmintic compounds. As such, we have developed and optimized a *Caenorhabditis elegans* liquid-based phenotypic assay to identify bacterially-produced secondary metabolites with anthelmintic activity. Although *C. elegans* are not parasitic, their ease of propagation and body plan similarity to parasitic nematodes makes them favourable for high-throughput compound screens. Using motility level readouts, we are able to search for molecules that paralyze or kill worms. From a semi-pure bacterial extract library, we have already identified two known potent inhibitors of *C. elegans* growth and development: Actinomycin D and Tunicamycin. Ongoing research involves elucidating the causative agent of impacted worm development from other screening hits. This paves the way for future work into isolating and characterizing bioactive molecules, which can ideally be optimized to treat helminthiases.

1221C **Pro-longevity compounds extend the lifespan and healthspan of *C. elegans* males** Rose S Al-Saadi[1], Patrick C Phillips-[21]Biology, University of Oregon, [2]University of Oregon

Aging is a universal phenomenon experienced by nearly all multicellular organisms. It represents the primary risk factor for multimorbidity and most chronic and neurodegenerative diseases. Despite this, knowledge of the molecular mechanisms that contribute to healthy aging remains limited. The nematode *Caenorhabditis elegans* has emerged as an important model for **identifying compounds that promote healthy aging**. While a number of compounds with beneficial effects on lifespan (and occasionally healthspan) have been observed in *C. elegans* hermaphrodites, only a few compounds have been tested on males. In order to develop *C. elegans* males as a novel screen for compounds that extend both healthspan and lifespan, we have treated males with a panel of pro-longevity compounds, utilizing lifespan assays to measure the effects on lifespan and mating assays to measure the effects on healthspan. Male mating behavior of *C. elegans* represents an excellent measure of healthspan because it is a robust, neurologically complex age-associated behavior. Males' consistent drive to mate significantly decreases over a

short period of time as they age, a consequence of neuronal health decline. Of the panel of compounds that have been shown to have positive effects on hermaphrodite lifespan, we find that only a subset also have positive impacts on lifespan and healthspan in males. This reveals a sex-specific impact of some of the previously published pro-longevity interventions. This project provides a new paradigm for screening and testing pro-longevity compounds in a new system and will provide a better understanding of the role genetic sex plays in influencing aging and age-associated decline.

1222C **The impact of *C. elegans* ceramide glucosyltransferase enzymes on the beneficial effects of *B. subtilis* on lifespan** Chelsey Arvin[1], Jason Chan[21]COM, Marian University, [2]Biology, Marian University

At the membrane surface of intestinal cells, the enzyme ceramide glucosyltransferase (cgt) catalyzes the addition of sugar molecules to ceramide, which plays a role in stress response and cell death. At this site of the intestinal lumen, bacteria can also colonize and impact host physiology, including lifespan. However, it is not known how bacteria-host interactions are affected by glucosylceramide metabolism and cgt enzymes. To test this, we asked whether worms with mutations in *cgt* enzymes would respond to the commensal bacteria *Bacillus subtilis. B. subtilis* has been found to increase lifespan and promote improved stress response in *C. elegans.* We examined whether the protective effects of *B. subtilis* require cgt enzymes (*cgt-1, cgt-2,* and *cgt-3*). For this, lifespans of wildtype (wt) were compared with *cgt* mutant animals grown on the wt *B. subtilis* isolate (3AIT), a mutant *B. subtilis* isolate that doesn't form biofilms, or the lab *E. coli* (OP50). Oxidative stress response was determined by examining response of 1-, 5-, and 10-day animals to100mM paraquat. The beneficial effect of *B. subtilis* for healthspan, such as stress response, was found to be dependent on CGT enzymes in 5-day old worms, mainly *cgt-1(ok1054*) and *cgt-3(tm504*). The results are not seen with 1-day nor 10-day old worms. These two genes demonstrate pro-survival roles in our studies.

1223C **Protective effect of sulforaphane on cadmium toxicity on the Caenorhabditis elegans model** Estefani Yaquelin Hernández Cruz[1,2], Dianelena Eugenio-Pérez[1,3], José Pedraza Chaverri[11]Biology, National Autonomous University of Mexico, [2]Postgraduate in Biological Sciences National Autonomous University of Mexico, [3]Postgraduate in Biochemical Sciences, National Autonomous University of Mexico

Cadmium (Cd) is a highly toxic heavy metal that causes serious health problems and even death. It is absorbed significantly by cigarette smoke, water, food, and air pollution. Mitochondrial dysfunction and alterations in redox homeostasis are involved in the Cd toxicity. Currently, existing therapies to treat toxicity due to this metal are based primarily on chelating compounds, which in the long run, have harmful effects. Therefore, finding solutions to relieve Cd-induced toxicity with fewer side effects is necessary. In this study, we investigated the protective effect of the sulforaphane antioxidant (SFN) on the toxicity induced by cadmium chloride (CdCl2) in the nematode Caenorhabditis elegans (C. elegans). Wildtype animals (N2 Bristol) on stage L1 were placed in medium K (51 mM NaCl, 32 mM KCl) with 1/10 of the OP50 strain of Escherichia coli and 100 µM of SFN for 24 hours at 20°C. Subsequently, without withdrawing the SFN, the CdCl2 was added to a concentration of 4,600 µM for another 24 hours at 20°C. We had groups treated only with SFN for 48 hours and CdCl2 for 24 hours. SFN decreases the toxicity of the CdCl2 by increasing survival (≈40%), body size, and lifespan of the nematodes. However, there is no statistically significant effect on lipofuscin levels. On the other hand, to study the effects of SFN on CdCl2-induced mitochondrial alterations, mitochondrial membrane potential was evaluated using the fluorescent probe JC-1 and the oxygen consumption associated with the mitochondria with high-resolution respirometry (Oroboros, Innsbruck, Austria). It was found that SFN restores the mitochondrial membrane potential and increases mitochondrial respiration compared to the group treated with CdCl2. Finally, to evaluate SFN protection with the nuclear factor erythroid 2-related factor 2 (Nrf-2) pathway (SKN-1 in C. elegans), one of the main targets of this antioxidant, the mutant strain GR2245 [SKN- 1(MG570)] was used. This strain was treated with the same scheme as the wildtype N2 strain. However, SFN was able to increase worm survival partially by 40%. These results suggest that SFN can decrease cadmium toxicity and mitochondrial disorders in C. elegans;
however, other pathways different to the SKN-1/Nrf-2 pathway are involved.

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1224C **Exploring Neuronal Preservation: Insights from *Caenorhabditis elegans* Dauer Diapause on Presynaptic Maintenance and Implications for Neurodegenerative Diseases** Sherlyn P Wijaya[1], Claire E Richardson[21]Laboratory of Genetics, University of Wisconsin-Madison, [2]Genetics, University of Wisconsin-Madison

The majority of human neurons have been present in our brains since embryogenesis, yet they have to maintain their complex morphologies and functions throughout our lifespan. As we age, our neurons inevitably deteriorate, possibly leading to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's. *Caenorhabditis elegans* challenges this 'inevitable' phenomenon through its alternative life stage – the dauer diapause. During this stage, worms maintain their neuron functions despite chronological aging, a clear contrast with non-dauer/aging worms at a chronologically-similar age. We thus aim

to harness the dauer state to uncover the genetic and cell biology mechanisms underlying extended presynaptic maintenance and discover why presynaptic maintenance fails with age. Our focus is directed toward extended presynaptic maintenance as it is central to preserving proper neuron functions, and yet it is hard to achieve due to the relatively long distance of presynapses from the cell body and their high metabolic demand. To address this issue, we first seek to characterize the effects of chronological aging on dauer presynaptic cell biology. For instance, in non-dauer *C. elegans*, chronological aging leads to a decline in synaptic vesicle numbers from the presynapse and accumulation of ectopic synaptic vesicles along the axon - does the dauer state prevent this? Then, we aim to identify which of the transcription factor(s) involved in dauer entry plays a role in extending presynaptic maintenance. We will use this knowledge to genetically de-couple the presynaptic maintenance phenotype from other dauer-related phenotypes. We have achieved cell-intrinsic suspension of neuronal aging within non-dauer/aging animals by manipulating a dauer-regulating hormone receptor in neurons – a phenomenon we called "dauerization." We will build on that and other upcoming results to discover which transcriptional target(s) of "dauerization" specifically instigate the long-term maintenance phenotype. By defining the mechanism behind presynaptic maintenance, we hope to lay the groundwork for understanding how presynaptic maintenance decline provokes the development of neurodegenerative diseases.

1225C **The Role of Transcription Factor FKH-9 in the *Caenorhabditis elegans* Oxidative Stress Response** Alessia Libertucci[1], Terrance Kubiseski[21]Biology, York University, [2]York University

Reactive oxygen species (ROS) is a highly reactive chemical that is generated from reduced oxygen molecules. ROS is formed in many different cell types from natural metabolic processes such as mitochondrial oxidative metabolism. This particular production of ROS is a normal cellular event; however, its accumulation can cause oxidative stress to the cell. For instance, cell exposure to environmental xenobiotics is one cause of ROS buildup. Biotransformation of these chemical substances known as xenobiotic metabolism, is needed for transportation and excretion out of the cell. However, this process is usually accompanied with the generation of ROS which can then go on to modify compounds, interfere with signalling cascades and damage macromolecules such as lipids, proteins, and DNA**.** Therefore, the cell is known to have multiple detoxification systems which can involve specific cellular signalling pathways. One pathway seen in *C. elegans* is the skinhead-1 (SKN-1) glutathione s-transferase-4 (GST-4) pathway. GST-4 is an important enzyme that helps to reduce the accumulation of ROS in the cell, while the SKN-1 transcription factor regulates *gst-4* at the promoter. However, under non-oxidative stress-inducing conditions SKN-1 is hypothesized to be indirectly held in the cytoplasm by BRCA1 associated protein-2 (BRAP-2). Recently, our lab discovered elevated *gst-4* levels in *brap-2* mutants, however RNAi of forkhead-9 *(fkh-9)* in these mutants caused a decrease in *gst-4* expression. This ultimately suggested a potential role that FKH-9 may play in the SKN-1 mediated oxidative stress response. However, the functional effects of FKH-9remain largely unknown. Therefore, we have taken initiative into examining how exactly FKH-9 may be affecting *gst-4*expression using qRT-PCR analysis and *gst-4::gfp* fluorescence imaging in both WT and mutant worms. The biological role of FKH-9 has also been investigated using both longevity and survival assays. Following this, luciferase assays have been carried out using human embryonic kidney (HEK) cells to aid in the determination of FKH-9's particular place in the signaling pathway. This analysis ultimately presents a substantial indication upon the role and function of FKH-9 in the oxidative stress response.

1226C **The Role of Transcription Factor, ZTF-22, in Oxidative Stress Response in *C. elegans*** Fozia Saleem, Terrance Kubiseski Biology, York University

Oxidative stress is caused when the balance between Reactive Oxygen Species (ROS) and detoxification enzymes is disturbed. In *Caenorhabditis elegans* (*C. elegans*), GST-4 is an important detoxification enzyme that is activated by the SKN-1 transcription factor when the cell experiences oxidative stress. Our lab previously showed that SKN-1 is negatively regulated by BRAP-2 and *brap-2* mutants show elevated levels of *gst-4.*

Our lab performed an RNAi suppression screen, using a transcription factor library, and found that the knockdown of another transcription factor, ZTF-22, also enhanced *gst-4* levels. Therefore, ZTF-22 was selected for further investigation in the context of oxidative stress. I found that loss of ZTF-22 caused an increased expression of *gst-4*, and *sod-3*. I also found that *ztf-22* deletion caused lifespan extension, reduced survival under heat stress and increased survival under the ER stress. Therefore, ZTF-22 contributes to the oxidative stress response, but also plays biological roles in thermotolerance, longevity, and the ER stress response.

1227C **Host-microbe interactions regulate mitochondrial function and lipid metabolism in *C. elegans*** Nathan G Dennis, Mireya Vazquez-Prada, Antonis Karamalegkos, Feng Xue School of Biosciences, University of Kent

Microbiomes profoundly affect host health, including metabolic, immune and nervous system functions. Due to the complexity of the human microbiome, model organisms such as Caenorhabditis elegans offer excellent tools develop a mechanistic understanding of host-microbe interactions. We have developed a model system consisting of C. elegans combined with an experimental microbiome isolated from wild C. elegans. The experimental microbiome colonises the C. elegans gut, producing a gut microbiome which diverges in composition from their bacterial food source.

Although worms fed the experimental microbiome exhibit reduced motility in young adulthood, they are protected against age-related motility decline and are resistant to Aβ toxicity, suggesting the experimental microbiome improves age-related health. The experimental microbiome alters mitochondrial networks in body wall muscle, without affecting mtDNA copy number, ATP levels or muscle morphology. Protection against age-related motility decline requires dynamin-related protein 1 DRP-1, which regulates mitochondrial fission, and the transcription factor ATFS-1, regulator of mitochondrial UPR. These findings show that interactions between microbes and host mitochondria can improve age-related motility. The experimental microbiome also induces major changes in lipid metabolism. RNASeq data and imaging of reporters show that the experimental microbiome downregulates fatty acid desaturation genes, indicating a switch of host metabolism to degrade fatty acids. Lipidomic analysis show that the experimental microbiome has a more complex fatty acid profile compared to OP50 and increases levels of the monounsaturated fatty acid oleic acid. Imaging experiments visualising using the lipid droplets and neutral lipids show that the experimental microbiome protects against age-related adiposity. Moreover, the Δ9 desaturase fat-6 and Δ6 desaturase fat-3 are required for the experimental microbiome to protect against age-related motility decline. Together these data show that the experimental microbiome alter multiple aspects of host physiology and protects against age-related motility decline by altering mitochondrial network dynamics and altering lipid metabolism.

1228C **Endosomal trafficking protein TBC-2 modulates stress resistance and lifespan through DAF-16-dependent and independent mechanisms** Bokang Ko[1,2,3], Annika Traa[1,2,3], Sonja K. Soo[2,3,4], Abdelrahman AlOkda[1,2,3], Christian E. Rocheleau[2,2,5], Jeremy Van Raamsdonk[1,1,2,3,51]Neurology and Neurosurgery, Research Institute of the McGill University Health Centre, [2]Metabolic Disorders and Complications Program, Research Institute of the McGill University Health Centre, [3]Brain Repair and Integrative Neuroscience Program, Research Institute of the McGill University Health Centre, [4]Neuroscience and Neurosurgery, Research Institute of the McGill University Health Centre, [5]Medicine, McGill University

The FOXO transcription factor, DAF-16, plays an integral role in insulin/IGF-1 signaling (IIS) and stress response. In conditions of stress or decreased IIS, DAF-16 moves to the nucleus where it activates genes that promote survival. To gain insight into the role of endosomal trafficking in resistance to stress, we disrupted *tbc-2*, which encodes a GTPase activating protein that inhibits RAB-5 and RAB-7. We found that *tbc-2* mutants have decreased nuclear localization of DAF-16 in response to heat stress, anoxia, and bacterial pathogen stress, but increased nuclear localization of DAF-16 in response to chronic oxidative stress and osmotic stress. *tbc-2* mutants also exhibit decreased upregulation of DAF-16 target genes in response to stress. To determine whether the rate of nuclear localization of DAF-16 affected stress resistance in these animals, we examined survival after exposure to multiple exogenous stressors. Disruption of *tbc-2* decreased resistance to heat stress, anoxia, and bacterial pathogen stress in both wild-type worms and stress-resistant *daf-2* insulin/IGF-1 receptor mutants. Similarly, deletion of *tbc-2* decreases lifespan in both wild-type worms and *daf-2* mutants. When DAF-16 is absent, the loss of *tbc-2* is still able to decrease lifespan but has little or no impact on resistance to most stresses. Combined, this suggests that disruption of *tbc-2* affects lifespan through both DAF-16-dependent and DAF-16-independent pathways, while the effect of *tbc-2* deletion on resistance to stress is primarily DAF-16-dependent. Overall, this work demonstrates the importance of endosomal trafficking for the proper nuclear localization of DAF-16 during stress and that perturbation of normal endosomal trafficking is sufficient to decrease both stress resistance and lifespan.

1229C **PRMT-7 wards the plasma membrane integrity compromised by bacterial pore-forming toxin through the activation of HLH-30/TFEB-dependent intrinsic cellular defense** Hui Chen Hsieh, Chang Shi Chen Institute of Basic Medical Sciences

Plasma membrane integrity is essential for cellular homeostasis. Bacterial pore-forming toxins (PFTs) that disrupt host plasma membrane integrity are required for the infection for various pathogens. However, how host cells guard the plasma membrane integrity in response to bacterial PFT intoxication remains obscure. In our previous study, we demonstrated that the HLH-30/TFEB-dependent intrinsic cellular defense (INCED) systems are activated, upon bacterial PFT intoxication, to repair the plasma membrane leakage in the intestinal epithelium of *Caenorhabditis elegans*. The transcription factor HLH-30/TFEB is a master regulator of lysosomal biogenesis and autophagy, yet the molecular mechanism for the full activation of HLH-30/TFEB elicited by PFT remains largely unknown. Here, we reveal that the protein arginine methyltransferase-7 (PRMT-7) is indispensable to the nuclear transactivation of HLH-30 in *C. elegans*. Our mass spectrometry and genetic analyses illustrated that PRMT-7 participates in the methylation of HLH-30 on its Rag GTPase binding domain to interrupt the LET-363/mTOR-dependent phosphorylation of HLH-30 and facilitates its nuclear localization. Moreover, we also showed that PRMT7 is evolutionarily conserved to regulate TFEB cellular localization and to repair plasma damages caused by bacterial PFT in human intestinal cell. Taken together, our data not only show a novel post-translational regulation of HLH-30/TFEB modulated by PRMT-7, but also sheds light on the evolutionarily conserved mechanism of the intrinsic epithelial host defense against PFT intoxication in metazoans.

1230C **Construction of a dauer-specific marker for the analysis of the dauer signaling pathway in *C. inopinata*, which has a low frequency of dauer formation** Ryo Iitsuka, Shun Oomura, Ryuhei Hatanaka, Asako Sugimoto Tohoku University

Across Nematoda, diverse environmental cues trigger the entering of the dauer state. The environmental and genetic factors that regulate dauer entry have been extensively studied in *C. elegans*, which readily enters the dauer state by starvation and high population density. *C. inopinata* is the closest sibling species to *C. elegans* that proliferates in fig syconia and uses a pollinating fig wasp as a carrier as dauer larvae. However, it rarely enters the dauer state in response to starvation under laboratory conditions, with a 0-1.7 % frequency (Hammerschith *et al*., 2022). To understand the diversity of dauer entry mechanisms, we performed a comparative analysis of dauer signaling pathways between *C. elegans* and *C. inopinata*.

To facilitate analysis of the dauer signaling pathway in *C. inopinata*, we established a dauer-specific marker strain that enables efficient detection of the dauer state in *C. inopinata.* A previous study in *C. elegans* reported that a collagen gene *col-183* could be used as a dauer marker, expressed in the hypodermis from the dauer-committed L2d through the dauer (Shih *et al*. 2019). We identified the *C. inopinata col-183* ortholog (*Ci-col-183*), constructed a transcriptional fusion gene *Ci-col-183p::mCherry*, and established an integrated *C. inopinata* line by microparticle bombardment. Similarly to *C. elegans*, *Ci-col-183p::mCherry* was expressed specifically in the pre-dauer and dauer states but not in other developmental stages, confirming that *Ci-col-183p::mCherry* can be used as a dauer marker.

Next, using this *Ci-col-183p::mCherry* strain, we assessed the function of some *C. inopinata* orthologs of the *C. elegans* dauer signaling pathway genes. *C. inopinata* orthologs were detected for most of the 53 *C. elegans* dauer signaling pathway genes, except for some GPCR and insulin ligand genes. We performed RNAi for some Daf-c orthologs at a higher-than-optimal temperature. Under this condition, *C. elegans daf-2(RNAi)* worms expressed *col-183p::mCherry* at a high level, but *C. inopinata daf-2(RNAi)* worms did not, raising the possibility that the dauer signaling pathway upstream the *col-183* may be at least partially different between *C. elegans* and *C. inopinata*. Other RNAi analyses for the Daf genes are currently in progress.

1231C **Hunting for the function of the excretory gland cell and NSPC proteins in worms** Zuzanna Mackiewicz[1], Vladyslava Liudkovska[1,2], Paweł S Krawczyk[1], Andrzej Dziembowski[1]1International Institute of Molecular and Cell Biology, [2]The International Institute of Molecular Mechanisms and Machines

The excretory gland cell is the most enigmatic part of the worm excretory system. Its function remains unknown, and laser-mediated ablation experiments performed more than 40 years ago did not reveal any obvious defects. In our previous work (*Liudkovska et al. Science Advances, 2022),* we have described cytoplasmic poly(A) polymerase TENT-5, which is essential for the proper innate immune response. This evolutionarily conserved enzyme polyadenylates and stabilizes mRNAs encoding secreted proteins, like antimicrobial innate immunity effectors in the worm intestine. Interestingly, the most prominent TENT-5 substrates represent mRNAs of the poorly characterized family of nematode-specific proteins (NSPC). NSPCs are predicted to be structurally very similar to some known neuropeptides in *C. elegans*, but their function remains unknown. It is noteworthy that *nspc* genes are expressed exclusively in the excretory gland cell, with exceedingly high mRNA levels. These observations motivated us to investigate the role of NSPCs and the excretory gland cell in worm physiology.

Our initial studies show that NSPCs might be responsible for the regulation of worms' appetite. NSPCs silencing with RNA interference method (RNAi) leads to increased uptake of bacteria, faster development, and a slightly shorter lifespan of worms. Surprisingly, higher bacteria consumption does not result in increased body size or lipid accumulation in the intestine. Performed transcriptome analysis revealed that, indeed, many genes involved in lipid metabolism in worms are dysregulated upon NSPCs silencing. Additionally, we observed alterations in the expression level of some proteins regulating the worm's defense response, which suggests that NSPC proteins might, similarly to TENT-5, take part in innate immunity processes in *C. elegans*. In parallel, we performed the optogenetic ablation of the excretory gland cell and observed that its removal also leads to the dysregulation of lipid metabolism-related genes. Following functional studies are ongoing.

Concluding, our data suggest that NSPC proteins and potentially excretory gland cell play a role in the regulation of lipid metabolism in *C. elegans*. However, the mechanism needs further investigation.

1232C **Links Between the Gut Microbiota, Innate Immunity, and Amyloid-β Toxicity in *Caenorhabditis elegans* Models** Laura M Freeman[1], Mireya Vazquez-Prada[1], Nathan Dennis[1], Feng Xue[1], Jessica Teeling[2], Marina Ezcurra[1]1School of Biosciences, University of Kent, [2]School of Biological Sciences, University of Southampton

Age is the biggest risk factor of Alzheimer's disease (AD) affecting 7.1% of people over 65, through the accumulation of the neurotoxic peptide amyloid-β (Aβ), formation of neurofibrillary tangles and neuroinflammation. In addition to chronic low-grade inflammation and immunosenescence, biological ageing is also accompanied by alterations in the composition and richness of the gut microbiota with increases in pathogenic and pro-inflammatory bacterial species. Similar compositional changes are observed in the gut microbiomes of AD patients but not of healthy age-matched controls, suggesting interactions between AD pathology, gut microbiota and ageing. Since systemic inflammation has been shown to increase Aβ load in rodent models and Aβ has antimicrobial properties *in vitro* and *in vivo,* the innate immune system may drive changes in Aβ toxicity as a result of

age-related compositional changes to the gut microbiota.

To study the relationship between the gut microbiota, innate immunity and Aβ toxicity we have established a model using transgenic *C. elegans* expressing human Aβ combined with a simplified experimental microbiome representative of the natural *C. elegans* microbiota. We have shown that animals cultivated with the experimental microbiome develop normally, indicating they are not dietary restricted and show suppression of Aβ toxicity in multiple models expressing Aβ in body wall muscle. Thioflavin S staining has shown that the experimental microbiome reduces the number of amyloid plaques observed in our transgenic *C. elegans* model, suggesting that host-microbe interactions with the experimental microbiome are protective. In contrast, host-microbe interactions with the pathogen *Pseudomonas aeruginosa* negatively impact the worm by increasing Aβ toxicity. Transcriptomic analysis shows the experimental microbiome upregulates genes related to immunity, and we are currently investigating immune-related mechanisms by which the microbiome protects against Aβ pathology.

1233C **Regulation of longevity by an mTOR/steroid signaling axis in C. elegans** Klara Schilling[1], Alex Zaufel[2], Birgit Gerisch[3], Tarek Moustafa[4], Adam Antebi[31]Molecular Genetics of Ageing, Max Planck Institute for Biology of Ageing, [2]Universitätsklinikum Düsseldorf (UKD), [3]Max Planck Institute for Biology of Ageing, [4]Medizinische Universität Graz

The mTOR pathway is an important player regarding cellular growth and aging. It is well established, that the modest inhibition of mTOR slows organismal growth and promotes longevity across taxa, including *C. elegans*. Work in mammalian cells suggests that the steroid cholesterol is a positive regulator of mTOR. However, the possible relation of mTOR, cholesterol signaling and longevity is not well understood.

In worms, cholesterol is converted to bile acid like steroids called dafachronic acids (DAs) through a series of enzymatic steps. DAs bind to the steroid receptor transcription factor DAF-12, which is the *C. elegans* homolog to the mammalian Vitamin D Receptor, Liver X Receptor, and the Farnesoid X Receptor. DAF-12 serves as a molecular switch to regulate developmental timing, dauer formation, as well as gonad signaling mediated longevity. We therefore wondered whether *daf-12*/DA signaling might interact with mTOR, using *raga-1* mutants as a genetic model of reduced mTOR signaling.

Interestingly, we found that null mutations of *daf-12* as well as mutations in DA biosynthetic enzymes abolish *raga-1*longevity, suggesting that reduced mTOR signaling acts through DA/DAF-12 signaling to promote longevity. Consistent with the idea of a regulatory cascade, we found that *raga-1* mutation results in dramatic upregulation of DA levels to promote DAF-12 transcriptional activity. Furthermore, *daf-12* mutation reverses some of the *raga-1* mutation effects when looking at transcriptomics and metabolomics data. We are currently testing the potential role of downstream target genes and metabolites responsible for longevity in this regulatory cascade. In sum, our findings demonstrate that mTOR and DAF-12 steroid signaling act in a unified pathway to regulate animal longevity and may reveal novel mediators of longevity in this context.

1234C **Kombucha Tea Consumption Restricts Lipid Accumulation Through Induction of Lipophagy** Rachel DuMez The University of North Carolina at Chapel Hill

Kombucha Tea (KT), a fermented tea with roots in traditional Chinese medicine, has surged in worldwide popularity due to purported health benefits. KT contains a symbiotic culture of yeast and bacterial species, many of which are considered human probiotics. The molecular basis of KT health benefits have yet to be thoroughly explored in any animal model. Here, we establish *C. elegans* as a model to query the molecular interactions between Kombucha-associated microbes (KTM) and the host. We demonstrate that worms have an established gut microbiome after consuming a KTM-exclusive diet that mirrors the microbial community found in the fermenting culture. Remarkably, animals consuming KTM display strikingly reduced lipid levels, yet develop and reproduce similarly to *E. coli*-fed animals. Critically, consumption of a non-fermenting mix of KT microbial isolates resulted in elevated fat accumulation, demonstrating that KTMs do not impair nutrient absorption and that metabolites produced only during fermentation may restrict fat accumulation. To identify the host metabolic pathways altered by KTMs, we performed RNA-seq on KTM-fed animals and found that three lysosomal lipase genes are substantially upregulated in these animals. These lipases, LIPL-1-3, have been previously shown to promote lipophagy via catabolism of lipid droplets. Consistently, KTM-fed animals display reduced levels of triglycerides and smaller lipid droplet sizes. We propose that KTM-fed animals are exhibiting a fasting-like metabolic state, even in the presence of sufficient nutrient availability, possibly through induction of lipophagy. Indeed, loss of core lipophagy related genes (*lipl-1* and *lipl-2*) lead to increased lipid accumulation in animals consuming KTM. Interestingly, though these mutants have increased lipid accumulation relative to wild-type animals, their fat levels were still lower than animals consuming the non-fermenting mix of KT microbes, suggesting there are likely additional pathways engaged by KTM consumption that restrict lipid accumulation. We hypothesize that KT microbe consumption reconfigures host metabolism though the modulation of core nutrient sensing pathways and their downstream effectors, including induction of lipophagy, to promote increased fat utilization. Elucidating the host metabolic response to KT consumption will provide unprecedented insight into how this popular fermented beverage may impact human health and inform its use in complementary healthcare plans.

1235C **Functional characterization of the mitochondrial 3-ketoacyl-CoA thiolase (*kat-1*) in *C. elegans* using comparative metabolomics** Marie Désirée Scheidt, Siva Bandi, Stephan H. von Reuss Institute of Chemistry, University of Neuchâtel

Mutation of the mitochondrial 3-ketoacyl-CoA thiolase (T02G5.7, *kat-1*) is known to affect *C. elegans* lifespan [1] and lipid metabolism [2]. However, *kat-1* function has long remained enigmatic. Here we describe a functional characterization of *kat-1* using a combination of comparative metabolomics, natural product isolation, and incorporation experiments with stable isotope labelled precursors.

Comparative metabolomics of *C. elegans* wild-type (N2) and *kat-1(tm1037)* highlighted a variety of novel compounds that are strongly enriched in *kat-1*. Their isolation and structure elucidation by NMR spectroscopy revealed a diversity of modular glucosides (MOGLs) that carry at least one tiglate moiety suggesting that *kat-1* is involved in tiglic acid metabolism. Incorporation experiments utilizing an *E. coli Δile Δleu Δval* mutant as bacterial food source that could be specifically enriched with either L-[U-$^{13}C_5$]-valine, L-[U-$^{13}C_6$,^{14}N]-leucine, or L-[U-$^{13}C_6$,^{14}N]-isoleucine highlighted *C. elegans* metabolites derived from branched chain amino acid metabolism. Tiglate units of the *kat-1*-enriched MOGLs were specifically [$^{13}C_5$]-enriched upon incorporation of L-[U-$^{13}C_6$,^{14}N]-isoleucine, indicating its function as a 2-methylacetoacetyl-SCoA thiolase in mitochondrial L-isoleucine metabolism. Free *anteiso*-C5 acids upstream of 2-methylacetoacetic acid, such as nilic acid and tiglic acid, were also [$^{13}C_5$]-labelled upon incorporation of L-[U-$^{13}C_6$,^{14}N]-isoleucine and strongly enriched in *kat-1*. An increased flux of *anteiso*-C5 building blocks from mitochondria was characterized by ESI-(+)-MS^E screening for *O*-acyl carnitines, which demonstrated that *O*-nilate-L-carnitine, *O*-tiglyl-L-carnitine, and *O*-2-methylbutyryl-L-carnitine are strongly enriched in *kat-1*. Furthermore, comparative metabolomics demonstrated that formation of tiglate substituted MOGLs is suppressed in *glo-1(zu391)*, *glo-3(kx94)*, and *glo-4(ok623)* mutants that are defective in proper formation of lysosome related organelles (LROs), which have previously been identified as sites for MOGL biosynthesis via carboxylesterase (*cest*) enzymes [3,4].

Taken together our results provide a functional characterization of *kat-1* as mitochondrial 2-methylacetoacetyl-SCoA thiolase and demonstrate that mitochondrial L-isoleucine metabolism provides tiglate building blocks that are exported via the carnitine shuttle and utilized in lysosome-related organelles for the biosynthesis of tiglate substituted modular glucosides. Whether and how this pathway results in the reduced lifespan of *kat-1(tm1037)* remains to be identified.

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1236C ***C. elegans* in rare disease diagnostics: association between *de novo FEM1C* variant and a neurodevelopmental disorder** Abhishek Anil Dubey[1], Magdalena Krygier[2], Natalia A Szulc[1], Karolina Rutkowska[3], Joanna Kosinska[3], Agnieszka Pollak[3], Malgorzata Rydzanicz[3], Tomasz Kmiec[4], Maria Mazurkiewicz-Beldzińska[2], Wojciech Pokrzywa[1], Rafal Ploski[31]Laboratory of Protein Metabolism, International Institute of Molecular and Cell Biology in Warsaw, [2]Department of Developmental Neurology, Medical University of Gdansk, [3]Department of Medical Genetics, Medical University of Warsaw, [4]Department of Neurology and Epileptology, The Children's Memorial Health Institute

Genetic defects in the ubiquitin-proteasome system (UPS), a principal component of the protein homeostasis network, are known causes of neurodevelopmental disorders. The exome sequencing of a pediatric patient with developmental delay, pyramidal signs, and limb ataxia showed an ultra-rare *de novo* missense variant c.376G>C; p.(Asp126His) in the *FEM1C* gene encoding a UPS enzyme - substrate receptor of a cullin-RING ligase complex. The identified variant alters a conserved amino acid located within the FEM1C substrate binding pocket. Our bioinformatic analysis confirmed its detrimental effect on the recognition of the C-terminus of SIL1 – the native substrate of FEM1C.

To further assess the pathogenicity of the mutation, we used the nematode *C. elegans* as a model of the patient's disease. We found that the FEM-1^{Asp133His} animals (expressing a variant homologous to the FEM1C p.(Asp126Val)) had normal muscle architecture yet impaired mobility, similar to the patient. Mutant worms were sensitive to the acetylcholinesterase inhibitor aldicarb but not levamisole (acetylcholine receptor agonist), showing that their disabled locomotion is caused by synaptic abnormalities and not muscle dysfunction. In addition, neuronal-specific RNAi depletion of FEM-1 also sensitized animals to aldicarb treatment indicating that FEM-1^{Asp133His} acts through the decreasing function of the protein. Our data suggest that inefficient turnover of neuronal substrates associated with the mutation in FEM-1 may lead to increased acetylcholine release, e.g., via impaired calcium sensing, overactivity of the machinery responsible for synaptic vesicle release, or excessive amounts of neuromodulatory peptides, resulting in aldicarb sensitivity, and motor and egg-laying defects.

In conclusion, we have identified a neurodevelopmental disorder associated with a novel FEM1C mutation that exerts functional consequences on the nervous system in a *C. elegans* model.

1237C **Promoting longevity using endogenous metabolites** Chieh Chen[1,2], Brett Lomenick[2], Min Chai[2], Wilson Huang[2], Jessie Chu[2], Laurent Vergnes[3], Randall M Chin[2], Xudong Fu[2], Karen Reue[3], Jing Huang[21]Molecular, Cellular, and Integrative Physiology Interdepartmental Program, University of California, Los Angeles, [2]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, [3]Department of Human Genetics, David Geffen School of Medicine at UCLA

Aging is a complex process that is directly related to human health and disease. The extraordinary finding that aging is malleable, as shown in model organisms whose life and health spans are extended by specific gene mutations or dietary or pharmacological perturbations, has offered enormous hope for our understanding and treatment of aging and related diseases. Although many molecules have been identified that can extend the lifespan of model organisms, few have been shown to alleviate age-related symptoms or illness in mammals. We have discovered several endogenous metabolites, such as α-ketoglutarate (α-KG), which when supplemented increase the lifespan of adult *C. elegans* and that of aged mice. We present new molecular targets including scaffolding proteins that we identified using an unbiased small-molecule target identification method, DARTS, combined with mass spectrometry, and discuss molecular and genetic mechanisms of longevity modulation by the metabolites. Targeting of broadly expressed scaffolding proteins in connection to cellular energy homeostasis seems to be a clever way that nature has devised for metabolite signals to impinge upon multiple organ and tissue systems, which may have utility for controlling aging and related diseases.

1238C **Sub-micromolar inhibition of whipworm motility by Auranofin, an approved human drug** Marina Nick[1], David B Sattelle[2], Frederick A Partridge[3], Kathryn Else[4], Ruth Forman[4], Carole Bataille[5], Angela Russell[51]Division of Medicine, UCL, [2]UCL, [3]University of Westminster, [4]University of Manchester, [5]University of Oxford

Almost half a billion people are infected by the whipworm *Trichuris trichiura*[1]. Current treatment of the infection, known as trichuriasis, is via preventative chemotherapy using the benzimidazole anthelmintic drugs albendazole and mebendazole. However, efficacy is low with single dose cure-rates of only 28% for albendazole and 36% for mebendazole. Hence the WHO roadmap for 2030 states that developing more effective medicines and drug combinations targeting *T. trichiura* is a critical action required to reach the target of eliminating trichuriasis as a public health problem[2].

Perhaps the fastest route to discovering candidate anthelmintic leads active against whipworm is to screen drugs already approved for human use. To this end the free-living nematode *Caenorhabditis elegans*[3] and the widely used laboratory experimental model for investigating whipworm, *Trichuris muris,* were deployed for screening purposes. We used an invertebrate automated phenotyping platform (INVAPP) to explore the actions of auranofin (MW 678), a triethylphosphine gold compound approved by the FDA for the orally administered treatment of rheumatoid arthritis in 1985. Auranofin has already been explored as a possible treatment for some nematode parasite infections. For example, in vitro it blocked the motility of filarial nematodes *Brugia* and *Onchocerca*, with sub-micromolar EC_{50} values. However, its limited *in vivo* efficacy may be due to its low oral bioavailability, a feature that could be advantageous for the treatment of a gastrointestinal tract nematodes such as *Trichuris*.

We show that auranofin is highly effective against the murine whipworm *T. muris* reducing whipworm motility with an EC_{50} of 6.0 x 10^{-7} M, a value comparable to the actions of commercial anthelmintics. Further research on auranofin›s actions on whipworm could potentially lead to a much-needed, anthelmintic to combat whipworm where there is an urgent, unmet clinical need.

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1239C **Exploring the Role of Dopamine in Adenylosuccinate Lyase Deficiency** Mia Peifer, Latisha Franklin, Corinna Moro, Wendy Hanna-Rose Biochemistry, Microbiology, and Molecular Biology, Pennsylvania State University

Inborn errors of purine metabolism are rare genetic disorders caused by mutations that alter the activity of key enzymes in both

purine biosynthesis and salvage. Adenylosuccinate Lyase Deficiency (ASLD) is one of these disorders caused by a deficiency in adenylosuccinate lyase (ADSL) activity. However, how these disorders arise on a molecular level is unknown. People who suffer from ASLD experience severe muscular and neurological symptoms. ASLD has been modeled by a deficiency of adsl-1 in the nematode C. elegans. One of the phenotypes this model displays is a defect in tyrosine metabolism. Previous research revealed that supplementation of tyramine, a metabolite derived from tyrosine, alleviates some of the phenotypes associated with an adsl-1 deficiency in C. elegans. Here, we show how dopamine, also derived from tyrosine, may help to alleviate some of these phenotypes.

Using the program WormLab, we characterized a defect in our adsl-1 deficient model's swimming behavior by measuring bending angles during one-minute periods of swimming. Control animals tightly regulated bending angles with the majority falling between 61 and 120 degrees. Our ADSL deficient model had a greater range of bending angles with the majority distributed evenly between 0 and 120 degrees. This data suggests that when adsl-1 activity is lost, regulation of bending angle while swimming is also lost. Considering our model's defect in tyrosine metabolism and dopamine's potential role in locomotion and swimming behavior, we hypothesized that dopamine supplementation, like tyramine, may also alleviate phenotypes associated with an adsl-1 deficiency. Upon supplementation with dopamine, bending angles of our adsl-1 deficient model returned to a distribution that more closely resembled control bending angles. This data suggests that dopamine supplementation may help to alleviate some of the swimming phenotypes associated with an adsl-1 deficiency. The results of these experiments may be useful for future development of treatments or therapies aiming to help those suffering from ASLD.

1240C **The interaction between the innate immune system and oxidative stress** Isabella Jensen, Alisa Liu, Jennifer R Powell Gettysburg College

All organisms are constantly exposed to potential pathogens. *C. elegans* employ multiple defense mechanisms against infection, including upregulation of infection response genes (IRGs), which encode antimicrobial proteins, upregulation of reactive oxygen species (ROS), and behavioral modification to limit subsequent pathogen exposure. Before these defenses can be activated, however, an infection must be detected by the innate immune system. Rather than detect pathogenic molecular patterns directly, the epithelial immune system is thought to monitor for indirect signs of infection such as cellular damage and stress. However, much is still unknown about the molecular mechanisms of innate immune recognition and response. *C. elegans* experience oxidative stress during infections as a result of the production of ROS by their own immune response and by ROS produced by the pathogens that are infecting them. We wondered whether cellular damage associated with oxidative stress may contribute to infection detection, with a focus on two overarching questions.

(1) Is oxidative stress sufficient to induce the innate immune response? To determine this, we measure mRNA levels of IRGs after exposure to an oxidant (paraquat) in the absence of infection using qPCR. To assess the behavioral response to infection, we train worms with nonpathogenic or attenuated bacteria in the presence or absence of an oxidant. After the training period, we determine whether the immune system associates oxidative stress with the nonpathogenic bacteria, leading to avoidance of a pathogenic strain during subsequent exposure. Finally, we will boost natural detoxification enzymes through a gain-of-function mutation in the master stress regulator SKN-1 and measure survival of wild-type and *skn-1(gf)* mutant worms in the presence of an oxidant.

(2) Is oxidative stress necessary to induce the innate immune response? To address this question, we measure the induction of the innate immune response upon infection with or without antioxidants to neutralize oxidative stress. We will confirm oxidative stress using fluorescent detox reporters and measure the immune response through qPCR of IRGs and behavioral avoidance of pathogens.

These experiments will help elucidate novel mechanisms involved in *C. elegans* innate immune recognition and response, and the implication of oxidative stress in the indirect detection of infection.

1241C **A biocompatible 3D printed microfluidic device for high-throughput C. elegans analysis** McKenzie E Garcia, Aaron Putzke, Philip Measor Biology, Whitworth University

Optimizing high-throughput analysis while balancing cost effectiveness continues to challenge C. elegans research. Worms are traditionally analyzed manually via microscopes or flow cytometers using phenotypes and fluorescence labelling, resulting in laborious and expensive experiments. More recently, microfluidic devices have been employed to analyze C. elegans quickly, but barriers to cost effectiveness remain. Alternatively, 3D printed (3DP) microfluidic devices offer an inexpensive, fast and highly adaptable method for analysis devices. Recently, a 3DP microfluidic device, consisting of poly(ethylene glycol) diacrylate, has shown favorable cytotoxicity for cellular growth, but the applicability to C. elegans had yet to be demonstrated.

Previously, we have demonstrated a biocompatible 3DP microfluidic device capable of analyzing C. elegans (T. Burchard, et al.,

SPIE Proc. 11955, 1195509, 2022). The internal tissues and green fluorescent protein were clearly visible through the 3DP microfluidic device. Microparticles (1 to 100 µm diameter) and worms were flowed through the device with speeds up to 500 µm/s using simple droplet pressure driven flow. At maximum speeds, up to approximately 180 nematodes per hour could be observed, imaged and analyzed.

In this work, we demonstrate movement of worms through a fully integrated 3DP microfluidic device using syringe-driven pressure with sufficient regulation to stop worms in a designated field of view and image at high resolution. Characterization the flow mechanics of vacuum pump versus syringe-driven flow via two types of interconnect ports at each end of the 3DP microfluidic channel were explored. A glass slide with microtubing or metal reservoirs with mounting wax were investigated. In both cases, generating flow using a closed system allowed for greater control of the pressure flow as well as a faster flow rate (up to 1133 µm/s) through the microfluidic 3DP device, while maintaining viability of the worms. However, highest efficiency of flow and pressure control were obtained with the glass slide/microtube interconnect in conjunction with the syringe driven flow. We demonstrate flow rates that allow for analysis speeds of up to 400 nematodes per hour is possible.

In this work, we demonstrated a novel, cost effective, biocompatible 3D printed microfluidic device with high imaging resolution and flow speeds offering a promising device for significantly increasing high-throughput analysis for biological studies.

1242C **An investigation into factors affecting *C. elegans* terminal investment** Noah Salewski[1], Dylan Kemmerer[1], Jennifer R Powell[21]Gettysburg College, [2]Biology, Gettysburg College

Environmental pressures affect every organism, and specialized response pathways are needed to react to each stressor. Most stress responses fall under one of two categories: somatic investment, a prioritization of the organism's own survival, and reproductive investment, an evolutionary strategy that enhances the survival of the organism's progeny. Terminal investment is a type of reproductive investment in which a stress is so severe and the provisioning of progeny so extensive that the death of the parent occurs but the progeny acquire resistance to future stress. *C. elegans* is an ideal model organism for studying stress, as they possess multiple specialized stress responses and myriad genetic tools to dissect these pathways. Additionally, *C. elegans* undergo terminal investment when exposed to acute hypothermia, characterized by a distinct loss of pigmentation. Nile Red lipid staining revealed that this phenotype is due to a massive reallocation of intestinal lipids into the germline.

We previously reported that the master stress regulator *skn-1* is important for cold-stress induced terminal investment. *skn-1* (*lax188)* gain-of-function mutations prevent lipid reallocation to progeny and promote survival of cold-shocked young adults. In contrast, Lynn et al (2015) reported that *skn-1(lax188)* promotes age-dependent somatic depletion of fats (Asdf), a movement of lipids from the soma into the oocytes of older adult worms. This led us to hypothesize an age-dependent role for *skn*-1 in lipid reallocation, and to question whether late-stage lipid localization could confer a survival benefit to embryos in the absence of adult cold stress. The *skn-1a* loss of function mutation *mg570,* but we are characterizing how the loss of additional isoforms affects the cold shock response.

We were also curious about the specificity of the *C. elegans* terminal investment response. Is the benefit of cold-stress-induced terminal investment a general resistance to stressors or specific to cold shock? Can other stressors trigger terminal investment, and if so, are the same molecular mechanisms used? Similar to cold-stressed worms, osmotically-stressed worms lose pigmentation; therefore, we tested whether osmotic shock could induce terminal investment. To determine whether cold stress provisions offspring specifically against future cold stress, we are testing whether the progeny of cold-stressed worms are resistant to extreme osmotic shock.

1243C **Enzyme kinetic characterization of wild-type IDH-1 and the G98N and R133H mutants** Melissa A. Bouchard, Anne McAllister, William C. Wolfe, Katherine M. Walstrom Div. Natural Sciences, New College of Florida

IDH-1 is the *C. elegans* ortholog of human cytosolic isocitrate dehydrogenase (IDH1), and both enzymes reversibly convert isocitrate to α-ketoglutarate (αKG). This enzyme is of particular interest because certain mutations that are associated with human cancers can reduce the normal activity of IDH-1 and introduce a neomorphic enzyme activity that converts αKG to 2-hydroxyglutarate (2HG). In order to determine if IDH-1 could be a reliable model system to study the cellular defects caused by IDH-1 mutations, we overexpressed and purified wild-type IDH-1 as well as two mutants, G98N and R133H, which correspond to the human G97N and R132H mutations, respectively. During our initial characterization of wild-type IDH-1 using spectroscopic assays to detect NADPH, we found that it has much higher activity in the presence of Mn^{2+} (K_M = 7 µM ± 1 µM) compared to Mg^{2+}, a strong preference for $NADP^+$ (K_M = 4.8 ± 0.7 µM) compared to NAD^+, and a maximum rate between pH 7.5-8.5. For the forward reaction, we determined K_M values for isocitrate of 6.9 ± 0.8 µM and 13 ± 1 µM and k_{cat} values of 2500 ± 70 min^{-1} and 189 ± 6 min^{-1} for wild-type IDH-1 and the G98N mutant, respectively, at 25 °C in 50 mM Tris-HCl, pH 8.0, 50 µM $NADP^+$, and 1 mM $MnCl_2$. We also determined a K_M for isocitrate of 4 ± 2 mM and a k_{cat} of 32 ± 6 min^{-1} for R133H at 25 °C in 50 mM Tris-HCl, pH 8.0, 5 µM $NADP^+$, and 2 mM $MnCl_2$. For the reverse reaction under the same reaction conditions, we determined a K_M for αKG of 14 ± 2

μM and a k_{cat} of 18.0 ± 0.7 min^{-1} for the R133H mutant. This is a lower K_M for αKG compared to the K_M values previously reported for αKG for the human R132H mutant. Our results demonstrate that wild-type IDH-1 and the R133H mutant have characteristics similar to the human wild-type IDH1 and its R132H mutant, respectively, in the forward reaction. However, the G98N mutant has a higher forward enzyme activity than the human G97N mutant. We are currently attempting to measure the amount of 2HG produced by the *C. elegans* enzymes in the reverse reactions.

(The first three authors had comparable contributions to this research project.)

1244C **Increased susceptibility to proteostasis collapse in C. *elegans* following consumption of UV-irradiated bacteria** Rachel Wellman[1], Annmary Paul Erinjeri[1], Germaine Wen[1], Shannon Barnes[1], Cassandra Backes[2], Filipe Cabreiro[2], John Labbadia[11]UCL, [2]Cologne Graduate School of Aging Research

The maintenance of protein homeostasis (proteostasis) requires the faithful synthesis, folding, trafficking and degradation of proteins and is crucial for proper cell function. A breakdown in the regulation of proteostasis leads to the accumulation and aggregation of mis-folded proteins, causing cell and tissue dysfunction. As cells age, their ability to maintain proteostasis declines, leading to the emergence of age-associated diseases including Alzheimer's, Parkinson's and cardiovascular disease. Understanding the factors that contribute to this decline in proteostasis collapse is crucial for the development of strategies to promote healthy ageing.

Recent work has demonstrated that the composition and activity of the gut microbiome is a crucial determinant of several aspects of health in both humans and *C. elegans*. However, the effects of bacterial stress on host proteostasis capacity are unknown. To explore this, we decided to investigate the interplay between microbiota stress and host proteostasis by observing the effects of bacteria that had been UV-irradiated (a common sterilisation method) on the ability of *C. elegans* to maintain proteostasis and suppress proteotoxicity throughout life.

We find that the consumption of irradiated *E. coli* during early adulthood increases the rate of proteostasis collapse and enhances polyglutamine induced proteotoxicity in neurons, muscles and the gut. We find that these effects are mediated by activation of the transcription factor NHR-49, which promotes the expression of metabolic and detoxification genes in response to the presence of UV-irradiated bacteria at the cost of host proteostasis capacity. We also find that this effect on the proteostasis capacity of *C. elegans* is dependent on the activity of rpe, lpp, lamB, ycgL and atpF genes in the *E. coli*.

Our work suggests that the presence of stressed bacteria in the gut has a negative impact on long-term proteostasis capacity in multiple tissues and that the use of UV as a sterilisation method could have important ramifications not only for long-term human and animal health human health (where UV irradiation is commonly used to treat water and food), but also for the use of UV irradiation as a method to "kill" bacteria in C. *elegans* research.

1245C **Healthspan enhancement after treatment with structured form of docosahexaenoic acid (DHA) in *C. elegans*** Ignasi Mora[1], Lluís Arola[2], Francesc Puiggròs[31]R+D, BrudyTechnology S.L., [2]Universitat Rovira i Virgili (URV), [3]Biotechnology Area, Eurecat, Centre Tecnològic de Catalunya

Although human lifespan has increased in the past century, the healthspan have not kept the pace, especially brain health. Among nutrients for maintenance of good brain function, the long-chain omega-3 polyunsaturated fatty acids (ω-3 LC-PUFAs): DHA and eicosapentaenoic acid (EPA), are the most promising ones. Recently, structured forms of EPA and DHA with enhanced bioavailability and bioactivity have been developed to improve their efficacy, and novel data have proven its powerful health benefits in comparison with conventional ω-3 supplementation.

Our research aims to study in more detail the effect of DHA on cognitive health while individuals get old. In this case, the treatment with a triglyceride form of DHA (DHA-TG) in aged (4, 8 and 12 days old) wild-type (WT) *C. elegans* showed a significant improvement of vitality through the thrashing assay, which is a well-known indicator of healthy ageing. Both lifespan and thrashing were measured with and without FUdR, an inhibitor of egg-hatching, to determine the impact that this drug could have on the nematodes, determining that differences in both parameters were larger under the FUdR effect. Despite any differences were found in sensitive cognition measured with the chemotactic assay, the treatment with structured DHA modified the antioxidant response of the nematodes through detection of lipid peroxides and glutathione, suggesting a correlation between motility improvements and antioxidant response in aged nematodes.

The innovative assessment of this study, which monitors performance and antioxidant parameters during aging, shows that supplementation with structured DHA-TG may improve the quality of life of healthy aged WT *C. elegans* and suggests novel mechanisms that need further studies.

1246C **Identification of natural antimicrobials using a *C. elegans* infection model** Georg Sandner[1], Kerstin Hangweirer[1], Lea

Karlsberger[1], Julian Weghuber[21]Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, [2]Center of Excellence for Foodtechnology and Nutrition, University of Applied Sciences Upper Austria

Pathogen infections play an important role in human and animal health as well as in food safety. Due to bans of antibiotic growth promotors or the pharmacological use of zinc oxide in the feed industry as well as increasing concerns regarding antibiotic-resistant bacteria, natural compounds (phytochemicals) gained more and more attention. Phytochemicals comprise secondary plant metabolites such as polyphenols, flavonoids or terpenes which were already shown to provide anti-inflammatory or anti-oxidant properties. In addition, common pathogens were identified to not only infect humans or higher animals but also *C. elegans*. The soil nematode possesses an innate immune system, thus capable of responding to bacterial infections. Hence, the use of *C. elegans* as an infection model provides a powerful tool for understanding host-pathogen interactions and developing alternative treatments for infectious human and animal diseases.

This work aims to identify natural anti-microbials by utilizing *C. elegans* as an infection model. Hence, *C. elegans* is infected with industry relevant pathogens including *Pseudomonas* or *Salmonella* and the course of infection under treatment with phytochemicals is observed.

Currently, suitable read-out parameters to monitor pathogen infection are validated. Multiple approaches including gene expression (immune response, anti-bacterial defence, oxidative stress), lifespan, lawn avoidance, fluorescent reporter strains or reactive oxygen species were evaluated in terms of reliable, robust, and fast response. So far, numerous target genes as well as fluorescent reporter strains were identified as interesting read-out parameters.

In parallel, a local plant library was screened for anti-microbial effects using a bacterial biosensor. Several plant extracts were successfully identified to provide anti-bacterial traits. Thus, infected *C. elegans* are currently treated with selected extracts to prove their anti-bacterial effect *in vivo*. Besides bacteriocidic effects, also increased susceptibility of pathogens to the host immune system or to classical treatments are of interest.

1247C **Using CRISPR knock-in of fluorescent tags to examine isoform-specific expression of EGL-19 in *C. elegans*** Kara McDonald, Ryan Doonan Glow Worms, The University of Texas at Austin

L-type voltage-gated calcium channels (VGCCs) regulate calcium influx and excitation-contraction coupling in many types of muscle cells. Thus, VGCC mutations can cause skeletal and cardiac muscle diseases in humans, including Duchenne muscular dystrophy and Timothy syndrome. To better understand the genetics and native expression of VGCCs, we have chosen to use the microscopic roundworm, *Caenorhabditis elegans*. The gene *egl-19* is the only L-type VGCC gene in *C. elegans*, and it encodes three distinct isoforms (a, b, and c). Isoform c is curious because the protein is very truncated, completely lacking the transmembrane domains that form the physical calcium channel. We have investigated *egl-19* by using CRISPR/Cas9 genome engineering to 'knock-in' fluorescent tags of differing colors that allow us to distinguish the expression of each isoform individually. Phenotypic analysis showed that these tags do not interfere with egg-laying capabilities, suggesting they are fully-functional tags. Not surprisingly, we found that all types of muscle cells express *egl-19*. Interestingly though, some neuron and muscle cells only seem to express the truncated c isoform. Furthermore, in muscle cells where more than one isoform is expressed, the isoforms appear to have unique subcellular distributions. This suggests that VGCC-dependent calcium influx might have localized functions and/or regulation in specific areas of a muscle cell.

1248C ***cnnm-5*'s role in the proteostasis of Huntingtin protein in *C. elegans*** Matt Hull[1], Will Singer[1], Gavin Graham[1], Joslyn Mills[21]Biology, Wheaton College - Norton, MA, [2]Biology, Wheaton College

Huntington's disease is a neurodegenerative disease characterized by the aggregation of mutant Huntington protein (mHTT). The severity of the disease directly correlates with the number of glutamine repeats expressed in the polyQ domain. The loss of proteostasis is believed to be the driver of the disease, so understanding how the two arms of proteostasis, autophagy and the ubiquitin proteasome system (UPS), are dysregulated is an important step to curing the disease. The *C. elegans* strain EAK103 is a model for Huntington's disease, in which a fragment of the human mHTT protein tagged with YFP (Htt513(Q128)::YPF) is expressed in the body wall muscles. EAK102 is a similar strain expressing the congruent fragment of normal human Huntingtin protein (Htt513(Q15)::YFP). In a small general RNAi screen, the knockdown of *cnnm-5* was identified to cause a decrease in mHTT accumulation compared to the no knockdown control (empty vector L4440) and was thus selected to further investigate.

The *cnnm* gene family encodes for membrane proteins essential for transporting magnesium to support gonadogenesis. While there is demonstrated redundancy within this family, the single knockdown of *cnnm-5* has offered a starting target for delineating its role in preventing or clearing mHTT protein aggregation. Our data demonstrates that *cnnm-5* knockdown upregulates autophagy and improves clearance of ubiquitinated proteins, suggesting *cnnm-5* could have a regulatory function in proteostasis. Our current efforts are focused on the effect of *cnnm-5* knockdown on lifespan and healthspan in the EAK103 strain and if the

specific clearance of mHTT can be enhanced. The magnesium transport function of *cnnm-5* and its role in regulating proteostasis is also currently being explored.

1249C **Indole-induced paralysis is not a stress-induced sleep state** Nikki Diya, Shantanu Bhatt, Matthew Nelson Biology, Saint Joseph's University

Immune function and sleep are interconnected; of no surprise, we sleep more when we are sick. Stress-induced sleep of *Caenorhabditis elegans* is a recovery sleep state which occurs as a direct response to exposure to noxious stressors, however pathogen-induced sleep has been largely underexplored. Enteropathogenic *Escherichia coli* (EPEC) are a major public health concern, having devastating impacts on children in developing countries. Additionally, EPEC is known to paralyze and kill nematodes, making *C. elegans* a powerful tool for assessing bacterial pathogenicity. However, the behavioral response of *C. elegans* during the infection process has not been extensively investigated. EPEC kills worms via two mechanisms: 1) Contact-independent which occurs following the release of indole, a bacterial toxin that causes paralysis; 2) Contact-dependent, following bacterial colonization of the intestine. We find that indole-induced paralysis is not a stress-induced sleep state. In fact, *ceh-17;aptf-1* double mutants, who have impaired ALA and RIS function, paralyze significantly faster than controls. Additionally, we find that increasing excitatory cholinergic input accelerates paralysis while increasing GABAergic input does not affect it. If worms are exposed to indole and then transferred to normal growth plates, they display stress-induced sleep that is ALA and RIS dependent. To better understand these mechanisms, we are conducting an EMS screen for suppressors of indole-induced paralysis.

1250C **Regulation of exopher formation in infertile *glp-1(gf)* mutant** Klaudia Kołodziejska[1], Agata Szczepańska[1], Nathalie Pujol[2], Michał Turek[11]Laboratory of Animal Molecular Physiology, Institute of Biochemistry and Biophysics Polish Academy of Sciences, [2]Centre d'Immunologie de Marseille-Luminy

Extracellular vesicles (EVs) are lipid-bilayer-enclosed particles that are released by most cell types. EVs are not only used by cells to remove unneeded biological material, such as misfolded proteins, they are also carriers for small molecules, proteins, and nucleic acids (among others) to be exchanged between cells. Recently, a new class of the large EVs, termed exophers, was discovered. It was shown that exopher generation is an evolutionarily conserved phenomenon found from invertebrates to mammals, including humans. Exophers were demonstrated to play a significant role in cellular stress response, tissue homeostasis, and organismal reproduction.

Attempting to unravel the regulation of muscular exopher formation in *C. elegans*, we recently observed exopher's release from muscle cells of sterile *glp-1*(gf) mutant which is characterized by increased germline proliferation. Knowing that exopher formation is sex-specific and fertility-dependent we are trying to solve this mechanism in an infertile animal. By comparing transcripts of *glp-1(gf)* mutants that do and do not produce exophers we were able to identify 114 genes differentially expressed between conditions. Among them, there are 24% of all known canonical intracellular pathogen response (IPR) genes. Moreover, we could demonstrate that both *glp-1(gf)* mutants and wild-type worms produce more exophers when exposed to the pathogen and our initial data demonstrate that this response is IPR-dependent. In conclusion, our findings suggest a cross-talk between exopher formation, cellular proliferation, and immune response which promotes worm›s better environmental adaptation.

1251C **The lipidomes of *C. elegans* with mutations in *asm-3*/acid sphingomyelinase and *hyl-2*/ceramide synthase show distinct lipid profiles during aging** Laphat Jean, Grace McIntyre, Melissa Guillen, Jason Chan, Trisha Staab Biology, Marian University

Lipid metabolism affects cell and physiological functions that mediate animal healthspan and lifespan. Lipidomics approaches in model organisms have allowed us to better understand changes in lipid composition related to age and lifespan. Here, using the model *C. elegans*, we examine the lipidomes of mutants lacking enzymes critical for sphingolipid metabolism; specifically, we examine acid sphingomyelinase (*asm-3*), which breaks down sphingomyelin to ceramide, and ceramide synthase (*hyl-2*), which synthesizes ceramide from sphingosine. Worm *asm-3* and *hyl-2* mutants have been previously found to be long- and short-lived, respectively. We analyzed longitudinal lipid changes in wild type animals compared to mutants at 1-, 5-, and 10-days of age. We detected over 700 different lipids in several lipid classes. Results indicate that wildtype animals exhibit increased triacylglycerols (TAG) at 10-days compared to 1-day, and decreased lysophoshatidylcholines (LPC). We find that 10-day *hyl-2* mutants have elevated total polyunsaturated fatty acids (PUFA) and increased LPCs compared to 10-day wildtype animals. These changes mirror another short-lived model, the *daf-16*/FOXO transcription factor that is downstream of the insulin-like signaling pathway. In addition, we find that *hyl-2* mutants have poor oxidative stress response, supporting a model where mutants with elevated PUFAs may accumulate more oxidative damage. On the other hand, 10-day *asm-3* mutants have fewer TAGs. Intriguingly, *asm-3* mutants have a similar lipid composition as the long-lived, caloric restriction model *eat-2*/mAChR mutant. Last, we use RNAi to knockdown lipid metabolic enzymes at different life stages and examen stress response after knockdown. Together, these analyses highlight the utility of lipidomic analyses to characterize metabolic changes during aging in *C. elegans*.

1252C **CBIOMES: establishing simple microbiomes for Caenorhabditis elegans space flight** Dana Blackburn[1], Adrien Assié[1], Estefania Torres[2], Daniela Vidal Vilchis[1], Nathaniel J Szewczyk[3], Monica Driscoll[4], Siva A Vanapalli[5], Buck S Samuel[11]Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, [2]SMART Program, 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, The State University of New Jersey, [5]Department of Chemical Engineering, Texas Tech University

As the frequency of humanity's travels to the stars grows, so does the urgency of understanding the impact of spaceflight on human biology. When astronauts travel to space, they do so together with billions of microbes in their bodies. Changes in microbiome form and function can tremendously impact host physiology from metabolism to stress resistance to predisposition to an ever-expanding list of diseases on Earth. Travel to space is accompanied by many changes in host physiology and the gut microbiome, but progress in understanding the relationship between host and microbiome has been hampered by human microbiome complexity and a previously limited pool of astronauts. To address these challenges, we are leveraging the robust, low-cost Caenorhabditis elegans spaceflight system to investigate microbiome impact on host physiologic responses to microgravity and the stress of space travel.

Previously, we have shown that C. elegans establishes a distinct gut microbiome that it acquires from its environment. Natural genetic variation of wild C. elegans strains on the scale observed between human astronauts can dramatically alter gut microbiome structure–i.e., the dominance of either Ochrobactrum (Alphaproteobacteria), Bacteroidetes, or Enterobacteriaceae. Building on these studies, we seek to establish a simplified and representative 3-member microbiome that recapitulates the dynamics of assembly and ultimate impacts on host physiology. To accomplish this, over 30 strains of bacteria (both alone and in mixtures) were subjected to a battery of growth conditions, assessments of viability and stability in culture, and profiles of gut colonization in wild and mutant C. elegans strains. Ultimately, a combination of Ochrobactrum BH3, Myroides BIGb0244, and Lelliottia JUb66 strains successfully model gut colonization of more complex communities.

Our work has demonstrated that complex community phenotypes can be recapitulated in a smaller community. Furthermore, this work provides a basis for our ongoing studies with C. elegans and its microbiome under microgravity conditions. These studies will be vital for understanding host physiology and health during long-term space travel as mankind prepares to navigate the moon, mars, and beyond.

1253V **Genetic and molecular features of *P. aeruginosa*-induced cleavage of *C. elegans* ribosomes** Alejandro Vasquez-Rifo[1], Victor Ambros[1], Denis Susorov[2], Andrei Korostelev[21]Molecular Medicine, UMass Medical School, [2]RTI, UMass Medical School

The bacterium *Pseudomonas aeruginosa* induces translation inhibition in multiple animal hosts by at least two routes. The first route is mediated by the post-translational modification of translation elongation factor 2 [1]. The second one relies on the cleavage of host ribosomes at helix 69 (H69) [2], a highly conserved rRNA hairpin critical for mRNA decoding and subunit joining. The ribosome cleavage strategy requires the bacterial quorum sensing system and host endocytosis, but the molecular mechanism of H69 cleavage is unknown.

Addressing this knowledge gap through genetic analysis, we screened candidate host and bacterial nucleases. We found that the loss of either *dis-3*, *pqe-1*, *disl-2*, *tsn-1* or *hoe-1* results in reduced levels of ribosome cleavage upon infection, suggesting that these genes contribute to ribosome cleavage either directly or indirectly.

Lysates from infected worms contain the activity that cleaves H69 [2]. This activity cuts H69 in *C. elegans* [2] and rabbit ribosomes but not in *E. coli* ribosomes. Also, the activity does not cofractionate with and is not competed by exogenously added H69 RNA hairpin. These results suggest that the H69 nuclease requires elements of the eukaryotic ribosome other than the target›s RNA sequence to elicit its activity. Finally, we have found that the activity is amenable to biochemical fractionation, as it elutes into single activity peaks when separated using size-exclusion, cation exchange, heparin column and ammonium sulfate precipitation approaches.

1254V **Contribution of fatty acid desaturation to Phosphine toxicity** Emma Firkins-Barriere, Paul EbertSchool of Biology, University of Queensland

Phosphine is a widely used fumigant for the control of insect pests of stored grain. Two Phosphine resistance genes have been found in pest insects, only one of which, *dld-1,* has been investigated in *Caenorhabditis elegans*. The other resistance gene is a fatty acid desaturase without a clear *C. elegans* orthologue. A screen of *fad* genes in *C. elegans* found that a null mutant of the

Δ6 fatty acid desaturase, *fat-3*, exhibits a strong phosphine resistance phenotype. Exposure to phosphine is known to increase the generation of reactive oxygen species that can peroxidise conjugated double bonds of the fatty acid tails of membrane lipids. We propose that the *fat-3* mutation decreases lipid peroxidation by decreasing the number of double bonds in membrane fatty acids. Raising wild-type C*. elegans* at 15°C, increases membrane desaturation to maintain membrane fluidity. We found that rearing wild-type *C. elegans* at 15°C, followed by equilibration to 20C and prior to fumigation at 20C, increased sensitivity to phosphine relative to controls of the same developmental stage that had been reared at 20C. We found that the *fat-3* mutant, like wild-type, was more susceptible to phosphine when reared at 15°C. This suggests that membrane desaturation in response to rearing at 15°C occurs independently of *fat-3*. This study highlights the contribution of cellular fatty acid desaturation to phosphine toxicity and presents a practical strategy to control phosphine resistant individuals via modulation of fatty acid composition of membranes with a 15°C temperature shift before fumigation.

1255V **CDC-48 influences SKN-1 activity in response to pathogen infection** Carolaing Gabaldonmicrobiology and molecular genetic, university of texas health science center at houston

In Caenorhabditis elegans, bacterial infections produce an imbalance in the amount of ROS in the cell, causing oxidative damage in molecules. Attempts to counteract the damage occur by transcriptional activation of detoxification programs in response to high levels of oxidative stress. In our lab, we observe the effects of infection on the host by exposing C. elegans to the human pathogens Enterococcus faecalis and/or Pseudomonas aeruginosa, which are ingested and colonize the lumen of the intestine.

The infection triggers the expression of the transcription factor SKN-1, a protein that is activated by ROS and is involved in the activation of detoxification genes such as gst-4 (glutathione Stransferase 4) and gcs-1 (glutamate-cysteine ligase), which encode proteins that promote the survival of the animal. An RNAi screen looking for genes whose loss prevented SKN-1 activation on pathogen discovered cdc-48. CDC-48 is involved in targeting ubiquitinated substrates for proteolysis and helps maintain cellular proteostasis. Specifically, loss of cdc-48 by RNAi failed to cause the activation of SKN-1 reporter genes following infection with E. faecalis or P. aeruginosa. Congruently, the levels of SKN-1 in the nucleus were observed to be significantly decreased. Additionally, the absence of cdc-48 during infection renders C. elegans significantly more susceptible to the pathogen.

My current focus is to understand the mechanism by which CDC-48 influences SKN-1 and this is an active area of ongoing investigation. In conclusion, CDC-48 affects the activation and nuclear localization of SKN-1 to affect survival on human pathogens such as E. faecalis and P. aeruginosa.

1256V **Biological activities of essential oils on *Caenorhabditis elegans*: from molecular targets to anthelmintic therapeutic strategies** Guillermina Hernando, Ornella Turani, Noelia Rodriguez Araujo, Cecilia BouzatInstituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Argentina.

Plants, herbal preparations and essential oils are used for health and medical functions since ancient times. Essential oils (EOs) have been extensively applied for human and veterinary health; their active agents have been isolated and incorporated into many current pharmaceutical preparations. Our study involves the use of *Caenorhabditis elegans* as a model of parasitic nematodes due to its simplicity and easy maintenance in the laboratory. We start from the hypothesis that EOs of aromatic plants used for aromatherapy or as food additives have anthelmintic action. We developed behavioral and molecular assays in wild-type worms, and analyzed mutant strains lacking receptors involved in locomotion to identify the pharmacological targets mediating the anthelmintic activities. We also explored the combination of current anthelmintics together with the addition of EOs purified compounds as a strategy to reduce resistance. In paralysis assays on agar plates, all tested EOs inhibited with different potencies *C. elegans* locomotion as well as egg hatching. The major compounds present in the tested EOs, trans-cinnamaldehyde (TC), geraniol, citronellol and linalool, were active at *C. elegans*. The combination of TC with the commercial anthelmintics levamisole and monepantel showed synergistic paralysis effects, while its combination with piperazine or ivermectin produced antagonic effects. Mutant worms lacking the levamisole-sensitive nicotinic receptor (L-AChR), GABA and glutamate receptors were partially resistant to these compounds. By single-channel recordings from *C. elegans* muscle cultured cells we deciphered how L-AChRs are modulated by TC. The analysis revealed that TC acts as an allosteric inhibitor of L-AChRs with key roles in nematode locomotion. Overall, we identified essential oils and their components as novel anthelmintic drugs and revealed their main pharmacological targets. Our results propose EOs as sources of natural compounds with promising polypharmacological profiles for anthelmintic therapeutics, and provide data on the efficacies of combinations that emerge as strategies to reduce drug resistance in nematodes.

1257V **Mechanism of oleic acid in alleviating violacein-induced toxicity in *Caenorhabditis elegans*** Jessica Antonio, Kyoung-hye YoonYonsei University Wonju College of Medicine

Oleic acid (OA) is an 18-carbon monounsaturated fatty acid that has been shown to alleviate numerous kinds of toxicity and

disease. Yet in some contexts it can induce toxicity. How OA carries out these effects is not fully understood. The effects of oleic acid can be observed even in the model organism, *Caenorhabditis elegans*. Among its known effects is alleviating the toxicity of a bacterial metabolite called violacein. Violacein inhibits the development of the worm, but adding oleic acid helps overcome this developmental arrest. Using this model, we performed a candidate RNA interference (RNAi) screen to uncover genes that are involved in mediating this beneficial function of oleic acid. We are currently exploring the signaling pathways of our genes of interest through additional genetic screens. Additionally, we have also found that OA benefit in violacein-treated worms require ROS, as antioxidants block OA-mediated growth. We will further characterize this hormesis effect, and try to identify how our candidate genes are involved in this process.

1258V **Phosphine toxicology of a field-evolved phosphine resistance variant using a CRISPR-generated *C. elegans* model** Taige Si, Paul EbertThe University of Queensland

Phosphine gas is the most effective and widely used fumigant against insect pests of stored grain, but increasing resistance to phosphine in the pest insects now threatens global food security. The P49S variant of the DLD enzyme, identified in the grain pest, *Rhyzopertha dominica*, is the most widespread phosphine resistance variant globally and has direct homologs that have independently arisen in several insect pest species. We have used CRISPR to edit the equivalent P>S variant (P39S) into the genome of the model organism *Caenorhabditis elegans* to use it as a phosphine toxicity model. We confirmed the phosphine resistance phenotype of P39S and evaluated the relative fitness against the near-isogenic wildtype strain, N2. We found negligible difference between the two strains in developmental rate and no difference in fecundity or heat-stress resistance. This apparent lack of a fitness cost of the mutation is consistent with the dispersal and persistence of insects that carry the resistance variant. Given that fumigation under hypoxic conditions is being proposed as a new fumigation strategy, we tested our model and found that low oxygen increases sensitivity to phosphine of the P39S strain. Interestingly, hypoxia increasing tolerance to phosphine of the wildtype strain, a result consistent with the response of phosphine sensitive insects. The net result of decreased resistance of the mutant and increased tolerance of the wildtype strain is that both strains are under equivalent selective pressure during fumigation.

1259V **CBIOMES Space Flight Project: Impact of the gut microbiome on the integrative physiology of genetically diverse *Caenorhabditis elegans*** Bushra Rahman[1,1], Purushottam Soni[1], Hunter Edwards[1], Atiyya Saroyia[1], Dana Blackburn[2], Girish Harinath[3], Monica Driscoll[3], Nathaniel Szewczyk[4], Buck Samuel[5], Siva Vanapalli[11]Chemical Engineering, Texas Tech University, [2]Molecular Virology and Microbiology, Baylor College of Medicine, [3]Rutgers University, [4]Ohio University, [5]Baylor College of Medicine

Recent space-flight studies in humans and rodents show alterations in the gut microbiome under microgravity, which adds a potential risk factor for flight crew health. Thus, it is crucial to understand the influence of the gut microbiome on long-term physiological changes in orbit for long-duration space missions. Previous spaceflight studies have not addressed gaps in understanding that include how differences between microbiomes contribute to changes in gut colonization, organ-level physiology, and whole-organism function under microgravity. Additionally, while studies on Earth have established the impact of individual genetic variation upon exposure to new environmental conditions, very little knowledge exists on how genetic diversity within individual species impacts the integrative physiology of organisms under microgravity. Most flight studies to date have focused on genetically homogenous rodent models or cell cultures.

Our CBIOMES project addresses these gaps by using recently established natural gut microbial communities to examine their impact on the physiology of *C. elegans,* and branches out to assess the host-microbiome interactions between genetically diverse animal strains. These studies investigate how the microbial colonization of the *C. elegans* gut contributes to host response, from the transcriptional level to whole organism behavior. We will present ground-study results with a 3-member community sampled from the *C. elegans* BigBiome.

The new knowledge and capabilities provided by this project will further microbiome-focused research in future spaceflight investigations, and may aid in the development of biotherapeutics for treating pathophysiological changes seen in space-flight and human disorders on Earth.

1260V **Investigating CSN-5's role in cancer development** Kellie Kuch, Ekaterina VoroninaUniversity of Montana

The COP9 signalosome (CSN) is a conserved eukaryotic complex regulating protein degradation via deneddylation of Cullin-RING E3 ligases (Qin et al., 2020; Wolf et al., 2003). CSN5, the CSN's fifth component, contains the catalytically active domain for CSN deneddylation (Echalier et al., 2013). The complex is inactive without CSN5; however, CSN5 undergoes CSN-independent binding with several other proteins, promoting either destruction or stabilization (Jin et al., 2014; Wei et al., 2008; Chamovitz, 2009; Orsborn et al., 2007). Of these, it's been shown to directly regulate tumor suppressors and proto-oncogenes, examples being p27 and MDM2 (Wang et al., 2016). The discovery of CSN5 upregulation in numerous cancers has led to the suggestion that

its dysregulation drives tumor formation and increased interest in it for cancer therapeutics (Jin et al., 2014; Pan et al. 2014). However, while upregulation has been documented, it has not been confirmed whether tumorigenesis is indeed secondary to CSN5 overexpression (Wang et al., 2016). Therefore, we seek to characterize CSN-5's role in tumor formation in the C. elegans germline. Our strategy is to generate worm lines with mutations increasing germline cancer susceptibility and containing a germline specific, CSN-5 overexpressing transgene; and then quantify proximal and distal germline tumor formation by visual scoring in parallel with microscopy and number of stem cell rows, respectively. Thus far, we generated gFLAG:csn-5; glp-1(ar202)gf and gld-2(q497); gFLAG:csn-5 lines. glp-1 regulates stem cell proliferation and maintenance in the germline, and the glp-1(ar202)gf line contains a temperature sensitive, gof mutation producing germline tumors (Pepper et al., 2002). gld-2 stimulates change from mitosis to meiosis, and the gld-2(q497) line has a lof mutation producing a shortened, inactive GLD-2 that when combined with a gld-1 mutant produces tumors (Hansen et al., 2004; Kadyk and Kimble, 1998). Comparison of gFLAG:csn-5; glp-1(ar202) gf and glp-1(ar202)gf at both 15°C and 20°C demonstrate no significant difference in tumor development, though do produce a significant increase in the number of distal stem cell rows at 20°C. These results call into question the hypothesis that upregulated CSN-5 leads to cancer, but do not discount its potential as a therapeutic. Moving forward, we will collect gld-2(q497); gFLAG:csn-5 data as previously described and generate a gld-1(q485); gFLAG:csn-5 line.

1261V **Revealing the link between lifespan in *Caenorhabditis elegans* and the mechanism of action of EAPB02303, a member of the Imiqualines family.** Perla Makhoul[1], Myriam Richaud[1], Carine Deleuze-Masquefa[1], Cindy Patinote[1], Raghida Abou Merhi[2], Hiba El Hajj[3], Simon Galas[11]Institut des Biomolécules Max Mousseron IBMM, UMR 5247, CNRS, ENSCM, Université de Montpellier, [2]Department of Biology, Faculty of Sciences, GSBT laboratory, Lebanese University, [3]Department of Experimental Pathology, Immunology and Microbiology, Faculty of Medicine, American University of Beirut

Caenorhabditis elegans has been extensively used to explore the aging process. While the regulation of this biological event is complex to decipher in humans, the molecular mechanisms are well characterized in the nematode. More importantly, molecular elements of the main signal transduction cascade involved in the worm lifespan regulation (Insulin/IGF-1 ILS pathway) have orthologous gene counterparts in humans (PI3K-Akt-FOXO). Thus, *C. elegans* is a great model to identify the age-dependent influence of novel molecules.

Imiqualines are a family of original molecules of low molecular weight synthesized by our team (F16 IBMM UMR5247) under international patent. The lead EAPB02303, displayed potent cytotoxic activity on a panel of cancer cell lines *in vitro*, with IC50s in the nanomolar range. In this study, we aim to identify candidate targets of EAPB02303 by using *C. elegans* to explore the impact of our molecule on main signaling pathways underlying the worm longevity.

Lifespan bioassay performed on the wild-type strain N2 showed a significant extension by 25% and 50% following treatment with EAPB02303 at 1µM and 10µM respectively. To unveil how EAPB02303 is prolonging longevity, we then looked at DAF-16, key transcription factor that integrates signals mainly from the ILS pathway and extends the nematode's lifespan via shuttling from the cytoplasm to the nucleus to activate age-modulating genes. Transgenic strain TJ356 expressing the fusion protein DAF-16:GFP was used to detect the cellular localization of DAF-16, and we measured a drastic increase in nuclear fluorescence from 12% under control conditions to 47% after exposure to EAPB02303.

Collectively, these results suggest a modulation of the ILS pathway and a subsequent activation of DAF-16 to prolong lifespan of *C. elegans*. The presented data provide a new insight on molecular targets of EAPB02303 underlying its potent anticancer activity, notably the pivotal transcription factor FOXO homolog of DAF-16, and the PI3K-Akt pathway, which is of particular importance since it is largely disrupted in cancer. Since aging has been previously linked to the reproductive state, analysis of the broodsize will be conducted to reveal the impact of EAPB02303 on fertility and development. Finally, we aim to perform an in-depth analysis using mutant strains for signaling pathways closely linked to several human cancers (*let-60* mutants homolog of human p21RAS) and main components of the apoptotic cascade (*Ced-9*, *Ced-3*).

1262V **AMPK signaling in swimming exercise mediated functional improvement** Sibaram Behera, Anindya Ghosh-Roy National Brain Research Centre

Functional decline is well documented with aging. Functional impairment is also observed after accidental injury in the human nervous system due to inefficient axon regeneration. Regeneration-associated functional recovery also declines with aging. Rehabilitative therapeutic approaches including physical exercise are a promising direction to improve functional recovery. Understanding specific downstream mediators of physical exercise might help to design a better therapeutic strategy. A single swimming session mimics the key features of the mammalian exercise in *C. elegans* (Laranjeiro et al., 2017).

Using the posterior lateral microtubule neuron (PLM) required for the posterior gentle touch sensation, we previously found that a single swimming exercise session of 90 minutes improves the posterior gentle touch function of day 5 stage worms which

is normally compromised due to aging. We also found that swimming exercise improves axon regeneration and associated functional recovery after laser-induced axonal injury (Kumar et al., 2021). The metabolic sensor AMPK/AAK-2 is a key mediator of the beneficial effects of swimming exercise (Kumar et al., 2021). Using the established aging and axotomy paradigms in PLM neurons, we tested several upstream and downstream effectors of AMPK signaling. Here we found that LKB1 serine /threonine kinase orthologue PAR-4, which phosphorylates AMPK upon depletion of ATP is also required for swimming-mediated functional improvement. Among the downstream effectors, we found that DAF-16 (FOXO) and MDT-15 (PGC1α) are required for the swimming-mediated positive effect. The beneficial effects of swimming exercise were not observed when PAR-4/AAK-2/DAF-16 axis was specifically removed from the body wall muscle using RNAi.This supports that the body wall muscle is a key hub regulating the swimming exercise effect. We are currently studying muscle-neuron communication in the context of swimming exercise using tissue-specific RNAi and rescue experiments.

References:

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1263V **Hormesis-like interactions between amyloid β and environmental stressors in *C. elegans*** James Lichty, Adriana San MiguelChemical and Biomolecular Engineering, North Carolina State University

Alzheimer's Disease (AD) is a neurodegenerative disorder which presents as a progressive loss of mental and physical function in those afflicted with it. AD is characterized by many abnormalities in the brain, such as widespread oxidative stress, immunity activation, neurodegeneration, inflammation, and protein aggregation. Amyloid β (Aβ) accumulation and aggregation has been associated with several AD traits, implicating it in the disease. To study this peptide's role in AD, several models using the organism *C. elegans* have been engineered to express Aβ pan-neuronally. Previous work has shown Aβ's ability to aggregate and induce oxidative stress, neurodegeneration, and reduced lifespan in *C. elegans,* but there are several other ways in which Aβ may influence the organism's health. To fully capture Aβ's impact on the organism and accurately translate it into a physiologically relevant result for humans, we need understand exactly what and how Aβ is interacting with the worm's own physiology. Environmental stressors, including heat stress, oxidative stress, starvation, and hypoxia, are known to significantly impact worm health, but their interactions with Aβ have been largely unexplored. This work focuses on elucidating how Aβ influences worm health and survival in response to several different stressors. We initially hypothesized that environmental stressors and Aβ would act synergistically to the detriment of worm health and survival. By exposing Aβ-expressing *C. elegans* to different stressors and scoring for survival, we show Aβ induces lowered oxidative stress resistance. Contrary to expectations though, Aβ expression increases resistance to several other stressors, indicating a possible hormesis-like effect. Additionally, we show, with a temperature inducible Aβ, a correlation between Aβ levels and heat stress resistance. Using gene-expression analysis techniques, we identified the *daf-16* pathway as a possible route for this interaction. This work aims to improve our understanding of how Aβ affects *C. elegans* to better design AD experiments and interpret the results in this model organism.

1264V **Elucidation of Factors Affecting Freeze-Thaw Tolerance in *C. elegans*** Naoko Sakai[1], Sawako Yoshina[1], Shohei Mitani-[21]Tokyo women's medical university, [2]School of Medicine, Tokyo women's medical university

Since the establishment of the *C. elegans* cryopreservation method (Brenner, 1974, Genetics), this method has supported many worm researchers worldwide to contribute to scientific development. *C. elegans* is unique among genetic model organisms in that it is a multicellular organism that can be easily cryopreserved. However, the mechanisms underlying the regulation of freeze-thaw tolerance in *C. elegans* remain poorly understood.

In this study, we examined several factors affecting survival rate after freeze-thaw conditioning in *C. elegans*. We found that starvation significantly increased freeze-thaw tolerance, which is consistent with the standard cryopreservation protocol that recommends freezing freshly starved worms. (Stiernagle, 2006, wormbook).

We also found that mutants of cuticular collagen genes, including *dpy-7* and *dpy-10* showed significantly reduced freeze-thaw tolerance. *dpy-7* and *dpy-10* mutants were reported to show abnormal localization of DPY-10 and DPY-7, respectively (McMahon et al. McMahon et al. 2003, Mol. Biol. Cell.). This result implies that these cuticular collagen molecules› proper distribution and function are crucial for *C. elegans*' ability to tolerate freezing.

Furthermore, we found increased freeze-thaw tolerance in *set-2*/SET1 and *wdr-5.1*/WDR5 mutants. SET-2 is H3-K4 specific histone methyl transferase, and WDR-5.1 is the conserved SET/COMPASS histone methyltransferase complex component. Both pro-

teins are responsible for depositing H3K4me3, suggesting that the methylation status of histones may be involved in regulating freeze-thaw tolerance in *C. elegans*.

Collectively, our findings suggest that three factors, namely starvation, precise cuticular structure, and histone methylation status, contribute to the regulation of freeze-thaw tolerance in *C. elegans*.

1265V **1-Mesytil-3-(3-sulfonatopropyl)imidazolium protects against oxidative stress and delyas proteotoxicity in *C. elegans*** Natalia Andersen[1], Tania Veuthey[1], Gabriela Blanco[1], Gustavo Silbestri[2], Diego Rayes[1], Maria Jose De Rosa[11]INIBIBB. CCT-UNS, [2]INQUISUR. UNS-CONICET

Due to the increase in life expectancy, age-related neurodegenerative diseases (NDs) have become more prevalent. Conventional treatments fail to arrest or delay neuronal proteotoxicity that characterizes these diseases. Due to their diverse biological activities, imidazole rings are intensively explored as powerful scaffolds for the development of new bioactive molecules. By using *C. elegans*, our work aims to explore novel biological roles for these compounds. To this end, we have tested the in vivo anti-proteotoxic effects of imidazolium salts. Since NDs have been largely linked to impaired antioxidant defense mechanisms, we focused on 1-Mesityl-3-(3-sulfonatopropyl) imidazolium (MSI), one of the imidazolium salts that we identified as capable of improving iron-induced oxidative stress resistance in wild-type animals. By combining mutant and gene expression analysis we have determined that this protective effect depends on the activation of the Heat Shock Transcription Factor (HSF-1), whereas it is independent of other canonical cytoprotective molecules such as abnormal Dauer Formation-16 (DAF-16/FOXO) and Skinhead-1 (SKN-1/Nrf2). To delve deeper into the biological roles of MSI, we analyzed its impact on previously established *C. elegans* models of protein aggregation. We found that MSI ameliorates β-amyloid-induced paralysis in worms expressing the pathological protein involved in Alzheimer's Disease. Moreover, MSI also delays age-related locomotion decline in other proteotoxic *C. elegans* models, suggesting a broad protective effect. Taken together, our results point to MSI as a promising anti-proteotoxic compound and provide proof of concept of the potential of imidazole derivatives in the development of novel therapies to retard age-related proteotoxic diseases.

1266V **The role of eIF3d in stress response** Jiaqing Lang Molecular and Cellular Function, University of Manchester, FBMH, School of Biological Science

Stress granules (SGs) are cytoplasmic ribonucleoprotein condensates that help reprogramme cells to adapt to and survive stress. Their dysregulation has been implicated in neurodegenerative diseases, cancer and ageing. SGs are conserved amongst eukaryotes and are composed predominantly of untranslated mRNAs, translation initiation complexes and RNA-binding proteins. They also interact with cellular signalling pathways to regulate changes in mRNA translation underpinning altered cell fate. The translation initiation factor eIF3d participates in canonical mRNA translation as a component of the eIF3 complex but can also directly bind to the 5' cap and recruit the rest of the eIF3 complex to a cohort of stress-responsive transcripts that promote cell survival. eIF3d and other eIF3 factors are also components of SGs and are required for their formation in cultured human cells. Furthermore, a pool of eIF3d is found in the nucleus, the function of which is unknown. These results hint at a role for eIF3d in coordinating different aspects of the stress response. However, much of our knowledge of stress-induced translational reprogramming as well as SG regulation and function are derived from cell-based studies, so it is important to understand this in the context of an organism. to We show that eIF3d and other eIF3 factors are required for SG formation in response to heat shock in *C. elegans*. We are now investigating how particular domains and post-translational modifications of eIF3d may play regulatory roles in the stress response and aim to uncover the relationship between SG function, eIF3d regulation and organism adaption over the lifespan of *C. elegans*. This research will provide new insights into the role eIF3d and SGs play in the integrated organismal response to both acute environmental insult and longer-term stress.

1267V **Effects of downstream mediators of DBL-1/BMP immune signaling on gut microbiome composition** Kenneth Trang[1], Siavash Karimzadegan[2], Barbara Pees[3], Michael Shapira[11]Integrative Biology, University of California, Berkeley, [2]University of California, Berkeley, [3]Evolutionary Ecology and Genetics, University of Kiel

The composition of the gut microbiome is determined by a complex interplay of diet, host genetics, microbe-microbe competition, abiotic factors, and stochasticity. Previous studies have demonstrated the importance of host genetics in community assembly of the *Caenorhabditis elegans* gut microbiome. More specifically, DBL-1/BMP/TGFb immune signaling has been shown to modulate microbiome composition, particularly affecting abundance of gut *Enterobacteriaceae*. Although *dbl-1(nk3)* and *sma-3(e491)* mutants, lacking the BMP-1-like ligand DBL-1 and its R-SMAD downstream transcriptional regulator, respectively, both exhibit a bloom of *Enterobacteriaceae* abundance, wildtype level control over *Enterobacteriaceae* abundance was primarily dependent on expression of *sma-3* in the epidermis, not in the intestine. This suggested that effects of Sma signaling on the gut microbiome were mediated by yet unidentified intestinal factors.

In the present study, we used RNA-seq gene expression analysis of wildtype, *dbl-1* and *sma-3* mutants, and a *dbl-1* over-expressing

strain raised on *E. coli* or on the CeMBio community to identify genes regulated by DBL-1/BMP signaling, particularly in response to a complex community. Following confirmation of several putative targets by qRT-PCR, we carried out colonization experiments with respective mutants raised either on CeMBio or on a second defined community, SC20. These experiments identified four intestinal Sma targets, three of them predicted to secreted, that showed increased *Enterobacteriaceae* abundance compared to wildtype. Current work is aimed at characterizing how these factors achieve that. The results presented expand the involvement of the Sma/BMP pathway in shaping microbiome composition to describe an underlying gene network that mediates its effects.

1268V **Lipid metabolism is regulated by SIR-2.1 through HSF-1 in *C. elegans*** Milán Somogyvári, Saba Tafiq Moqbel Khatatneh, Csaba Sőti Dept. of Molecular Biology, Semmelweis University

Obesity is a risk factor of several leading causes of death in our modern societies. Many of the hallmarks of obesity and metabolic syndrome have large overlaps with hallmarks of another important risk factor for various diseases: ageing. The SIR2 deacetylase has been widely proven to be a regulator of lifespan and energy metabolism. In our previous work we found a conserved chaperone client interaction between SIR2 and the abundant heat shock protein HSP90 – with a role in lifespan and fat storage regulation in mammalian cells and worms, which suggested an association with heat shock response and proteostasis. Here we confirm that SIR-2.1 is required for lipid mobilization in C. elegans: we found that loss and gene silencing of SIR-2.1 hinders starvation induced lipolysis. Besides this, we identified the heat shock transcription factor HSF-1 as the mediator of the observed inhibitory effect, taking place in the intestine, by modulating the expression of lipases, such as ATGL-1. Additional results show that HSF-1 utilizes the microRNA gene silencing system to modulate lipase expression, while acting independently from the PKA pathway, which is also involved in lipolysis-regulation. Uncovering the mechanism by which these actors are involved in lipid mobilization and lifespan determination brings us closer to answers that might help to fight some of the most pervasive threats to human health in the XXI. century.

1269V ***Caenorhabditis* Intervention Testing Program: Updates on robust longevity effects of novel compounds in genetically diverse nematodes** Brian Onken[1], Christine A Sedore[2], Anna Coleman-Hulbert[2], Stephen Banse[2], David Hall[3], Erik Johnson[2], Grace Jackson[2], Erik Segerdell[2], Hadley Osman[3], Elena Battistoni[3], Yunpeng Xu[1], Madhuri Achanta[1], Yuhua Song[1], Monica Driscoll[1], Gordon Lithgow[3], Patrick Phillips[2], Viviana Perez-Montes[4], Tiziana Cogliati[41]Rutgers, The State University of New Jersey, [2]University of Oregon, [3]Buck Institute for Research on Aging, [4]National Institutes of Health

Many efficacious interventions that promote mouse longevity (sirtuins, TOR, insulin signaling) have identification roots in invertebrate genetics. The Caenorhabditis Intervention Testing Program (CITP) was charged by the NIA to evaluate pharmacological interventions that promote healthy aging in a robust and reproducible manner across diverse genetic backgrounds of natural variant Caenorhabditis strains. The central premise of the CITP effort is that compounds that have strong effects across diverse genetic backgrounds should have enhanced probability of translatability into pre-clinical research. Indeed, some CITP-verified compounds have been shown to promote healthspan and longevity in mouse models, in support of this fundamental premise.

Our current effort includes pursuing compound submissions from the larger scientific aging community, as well as identification of candidates via high-throughput screening of chemical libraries and data mining of peer-reviewed publications. We now evaluate whole-organism RNA sequence data to estimate the mode of action for successful interventions, and conduct mortality analysis from a generalized family of distributions on high-resolution automated lifespan data to determine whether a given intervention changes the rate or onset of aging. We are also creating multi-species “at risk” test sets, in which components of aging hallmarks (such as proteostasis, stress response circuits) are genetically compromised in diverse genetic backgrounds. Such genetic test sets might expand understanding of intervention action and help rank compounds for translational testing.

Recent promising CITP longevity interventions include a small molecule, a proprietary compound, an isothiocyanate and a vitamin derivative. In addition, we find that metabolic modulator Metformin and anti-amyloid Thioflavin T, which promote longevity and healthspan in multiple test CITP strains, can positively impact lifespan in diverse genetic backgrounds in which proteostasis is compromised, suggesting wide-ranging health benefits of these potent pro-longevity compounds. We will present on the breaking compound successes.

1270V **Glycolytic flux is rewired in NAD^+ biosynthetic mutants** Tatyana Hollingbird[1,2], AbdulKareem AlShaheeb[2], Melanie R. McReynolds[31]Alcorn State University, [2]Pennsylvania State University, [3]Biochemistry and Molecular Biology, Pennsylvania State University

Nicotinamide Adenine Dinucleotide (NAD^+) is an abundant and critical molecule to the life of single-cell organisms like bacteria to multicellular organisms. This molecule is vital to the function of the body’s mitochondria. NAD^+ helps protect the body from disease and aging, as well as convert food into energy, in this case glucose. The biosynthesis of NAD^+ has been shown to be an engaging and promising therapeutic earmark for altering obesity-related, health-span, and tumor growth phenotypes. However,

NAD^+ is also a key to metabolite which impacts the metabolome and key signaling reactions. With knowing this, it is essential to clarify exactly how engineering NAD^+ biosynthetic pathways can lead to therapeutic benefits. Past research has revealed that glycolysis is in fact impaired in NAD^+biosynthetic mutants, which triggers reproductive and developmental phenotypes. Taken together, my work aims to answer the following questions: How is glycolytic flux rewired in all NAD^+ biosynthesis mutants in *C. elegans*? How does NAD^+ metabolism control reproductive aging in *C. elegans*? To this end, my research examined metabolic dysregulation in NAD^+ biosynthesis mutants, *pnc-1 (pk9605), kynu-1 (tm4924), and nmrk-1(ok2571)*, using stable isotope tracers and liquid chromatography mass spectrometry to investigate glycolytic flux when NAD^+ is limiting. To gain a deeper understanding of the core mechanisms behind NAD^+ salvage synthesis and glucose metabolism, I decided to directly test this model via application of metabolic isotopic carbon tracing tools. Metabolic carbon tracing confirmed that glycolysis is blocked in *pnc-1* mutants and enhanced in *kynu-1* mutants. Additionally, I observed an increase of isotopic label from glucose entering UDP-Glucose in the *nmrk-1* mutants. Metabolic carbon tracing provided key insight into elucidating compromised glycolysis due to loss of NAD^+ biosynthesis in *C. elegans*.

1271V **Host- and microbe-derived β-branched fatty acids regulate NHR-49/PPARα in *C. elegans*** Bennett W Fox[1], Maximilian Helf[1], Amaresh Chaturbedi[2], Alexander Artyukhin[1], Sylvia S Lee[2], Frank Schroeder[1]1BTI/Cornell University, [2]Cornell University

Nuclear receptors (NRs) are ligand-gated transcription factors and central regulators of metazoan physiology. Binding of cognate ligands to NRs orchestrates essential gene expression programs, but the identities of the small-molecule ligands of many NRs remain unknown. Here we show that endogenous and microbiota-derived metabolites promote lipid desaturation via NHR-49/PPARα in *C. elegans*. Untargeted metabolomics revealed an endogenous β-methyl fatty acid, bemeth#1, that potently activates NHR-49-dependent expression of the fatty acid desaturase *fat-7*. We show that bemeth#1 is derived from a methyltransferase, *fcmt-1*, that is conserved across Nematoda and likely originates from bacterial cyclopropane synthase via horizontal gene transfer. Parallel investigations into hyperactive NHR-49 signaling in mutants of the acyl-CoA dehydrogenase, *acdh-11*, revealed a β-cyclopropyl-fatty acid, becyp#1, whose structure and activity mimic the endogenous bemeth#1, but which originates from bacterial cyclopropane lipids. Elimination of cyclopropyl lipids from the bacterial diet abolishes becyp#1 production and rescues increased *fat-7* expression and temperature-dependent lethality of *acdh-11* mutants, demonstrating that accumulation of a bacterial lipid-derived NHR-49 agonist underlies the *acdh-11* phenotypes. Collectively, we demonstrate that evolutionarily related lipid biosynthesis pathways in metazoan host and associated microbiota converge on NHR-49/PPARα to regulate host fat metabolism.

1272V **The role of AHR signaling in ROS and microbiome regulation** Ciara Hosea, Buck Samuel Baylor College of Medicine

The gut is comprised of a diverse community of bacteria, or microbes, collectively known as the gut microbiome. The gut microbiome has been a topic of interest as of recent years, as this environment has been proven powerful enough to affect host health and physiology once disturbed. Partnerships between animals and their resident gut microbiomes are common and rely on specific mechanisms of communication to maintain homeostasis. Left unchecked, persistent microbial disturbances ('dysbioses') can have pathologic consequences on host development and physiology across organ systems.

A potential mediator of this partnership would be the aryl hydrocarbon receptor (AHR), which is a transcription factor that has previously been implicated in the maintenance of protective intestinal barriers, but more recent studies have provided a role for AHR in altering the species of bacteria present in the gut microbiome and metabolome. Though a role for AHR signaling in microbiome interactions has been revealed, current works lack clear molecular connections to explain the remodeling of the types and abundance of microbes present in the gut microbiome.

To address this, we utilized *C. elegans* and a defined 63-member natural microbiome (BIGbiome). Through the use of 16S profiling, we have found that mutants of the *C. elega*ns AHR ortholog, *ahr-1*, display an altered gut microbiome when compared to wild-type as in other systems. In *C. elegans*, *ahr-1* is a transcription factor that is expressed in a subset of oxygen sensory neurons within the animal. Seeing as, we chose to observe the role of reactive oxygen species in this interaction. When fed BIGbiome, *ahr-1* mutants displayed a general heightened presence of reactive oxygen species. This assay also revealed that certain microbes were acting in an *ahr -1*dependent manner, while others were acting in an *ahr-1* independent manner in relation to the amount of reactive oxygen species present and detected. We also profiled individual microbes to observe their sensitivity to and ability to produce H_2O_2. We aim to uncover the molecular mechanisms and networks used by *ahr-1* to contribute to its management of gut microbiome composition, host oxidative stress, and the possible molecular link between the phenotypes observed.

1273V **High-Throughput Analysis of New Organoselenium Compounds: Investigating Their Toxicity and Stress- Protective Properties in *C. elegans*** Natalia S Jardim[1], Daiana S Avila[2], Alexandre Benedetto[1]1Lancaster University, [2]Universidade Federal do Pampa

Selenium is an essential micronutrient for bacteria and mammals. Adequate selenium intake has been shown to be critical for

proper immune function - including against viruses such as SARS-CoV-2, resilience to cancers, and decreased risk of cardiovascular diseases. The chemical properties of this trace element have also attracted interest in organic synthesis for the development of catalysts and pharmacological agents.

Organoselenium compounds are synthetic molecules with a wide range of promising pharmacological properties including chemopreventive and antioxidant activities. However, their pleiotropic effects and high reactivity at sub-micromolar doses are also associated with toxicity risks, and their mechanisms of action are ill-defined.

In this study, we investigated the toxicity and stress-protective properties of 13 synthetic organoselenium compounds in C. elegans. We first employed a high throughput survival assay to assess compound ability to promote thermotolerance across a range of concentrations and dietary conditions, contrasting their efficacy to that of analogous non-selenide chemicals. Notably, we compared the effects of these organoselenides on worms fed live versus PFA-killed bacteria to identify direct and gut bacterium-mediated effects . We then measured life traits and health parameters of C. elegans for the most promising treatment paradigms.

Our results show that organoselenide compounds exert dose-dependent beneficial or detrimental effects on C. elegans health depending on nutritional status and gut microbe metabolic state. Dietary and gut microbiota changes accounted for most of the measured impact on thermotolerance. Yet, we identified significant additive health promoting effects between defined dietary changes and nanomolar exposure to specific organoselenides.

This work exemplifies how taking into account gut microbial activity and composition, organoselenides may be exploited to deliver health benefits.

1274A **Increasing undergraduate access to research with *C. elegans*** Joslyn MillsBiology, Wheaton College - Norton, MA

Hands-on learning is essential for students to fully grasp complicated concepts, but a single exposure to a technique or idea during a semester is usually not enough to achieve long-term retention of understanding. Students must be engaged in interactive learning in order for them to more successfully retain the concepts, increase attention, and have meaningful experiences which leads to significant learning. Further, success in the STEM fields correlates with exposure to research experiences *before* the period of attrition from the STEM majors. With this approach in mind, I designed a Course-based Undergraduate Research Experience (CURE) course to be offered to all Biology students that only need introductory biology to register. This course brings discovery-based research into the classroom to foster technical and conceptual learning, either as an introduction to or to increase retention of students in STEM fields. It provides the hands-on laboratory and project design experience students need to become more competitive to join a research lab for their eventual thesis work; particularly for those students that have not had the advantage of working in a lab before (minorities, females, etc). Students with prior experience in high school are typically favored, thereby limiting inclusivity and diversity. Therefore, the long-term goal of this course is to expose many students from a variety of backgrounds to research and help them meet curriculum requirements in support of the academic programs and associated labs and prepare them for future exciting STEM careers.

In the course, the students take on the role of a scientist, where they perform a reverse genetics screen in *C. elegans* to select a gene to explore for the rest of the semester. After selecting their gene, as a small group, they develop their own hypothesis and write and execute a proposal to test it using published literature to support their ideas, ending with a written report and oral presentation of their findings. This approach allows us to gather clues to what the function of a gene is, and we can further determine if this gene would be a potential target to design therapeutics to treat diseases. This CURE course is easily adaptable to be offered at other institutions and has been executed in traditional (fully in-person), fully online, and hybrid formats with great success.

1275A **Now worm proteins are the ones that are cool** Katherine M. Walstrom Div. Natural Sciences, New College of Florida

For the last ten years, my Biochemistry Lab course was a Course-based Undergraduate Research Experience (CURE). Each year, students were given a choice of 4-5 *C. elegans* metabolic enzymes to work on. Each group subcloned the cDNA for their chosen enzyme into a protein expression plasmid and purified the enzyme during the first half of the course. During the rest of the semester, the students designed research projects to perform with their enzyme (if it was soluble and had detectable activity). Most of the projects were eventually developed into an undergraduate thesis project, which is required for all students at our institution. These projects involved enzyme kinetics, and some tested protein stability (MDH-1 and MDH-2) or the effects of site-directed mutations. Some enzymes were orthologs of proteins involved in human diseases (GSPD-1 and IDH-1). The procedures we developed could easily be adapted for other lab courses or for other undergraduate research projects. I am also looking for someone who could use the plasmids we've created because only some of the enzymes have been described in journal articles.

(The title is an homage to The Guild.)

1276A **A worm hunting based workshop to create a cycle of STEM research awareness among first generation college students** Emily M Morgan[1], Serena J Meadows-Graves[1], Amanda Haio[2], Aaliyah Ringor[2], Venus Ghani[2], Robert J Luallen[11]Biology, San Diego State University, [2]San Diego State University

First-generation college students face many challenges and encounter financial, social, academic, and psychological struggles when trying to adapt to their new learning environment. In fact, only 20% of first-generation students graduate with their bachelor's degree and about one in three drop out of college within their first three years. With first-generation undergraduates already being at an extreme deficit, typically, awareness and accessibility to STEM research opportunities are stunted. This long-term effect will continue the cycle for lack of diversity in STEM.

Creating more obtainable and beneficial opportunities within college campuses can help promote science to students who have not been exposed to research. We sought to create a cycle of STEM research awareness among first generation, early-stage undergraduates through a 2-fold process. First, we created a lab-based workshop to hunt for wild *Caenorhabditis* nematodes and their associated microbiota to promote interest in scientific research among this population of undergraduates at SDSU to help assist them gain primary research experience while achieving their bachelor's degree. In this workshop, local first-generation undergraduates searched for wild nematodes, discovered microbial species that interact with these nematodes using fluorescence in situ hybridization (FISH), and identified them using PCR and metagenomics. Second, these workshop undergraduates developed curriculum based on their research and presented at local high schools, progressing awareness for STEM research among young students. As such, the importance of STEM research can be displayed to younger generations by reaching high school students and exposing them to the scientific research being conducted by students that look like them. Advancing STEM research opportunities at SDSU will help eliminate persistent psychological barriers about the accessibility of STEM research especially among first-generation college students and those from under-served communities.

1277A **Using CRIPSR Engineering in an Upper-level Biotechnology Class to Model a Neurodegenerative Disease** Amy C. Groth[1], Rachel Ulitsch[1], Luis Flores-Gomez[21]Biology, Eastern Connecticut State University, [2]Physical Sciences, Eastern Connecticut State University

The relative ease and cost-effectiveness of working with *C. elegans* has led to widespread use of the species both as a model of human disease and in undergraduate laboratory classes. We attempted CRISPR genetic engineering in a small, upper-level biotechnology course to create a model of one of the human spastic paraplegias (HSPs). We chose HSP35, now included in the classification of fatty acid hydroxylase-associated neurodegeneration (FAHN). The human disease is autosomal recessive (caused by mutations in fatty acid 2-hydroxylase (FA2H)) and the worm ortholog, *fath-1*, is carried on an autosome (chromosome I). Due to the recessive nature of the human disorder and published research that *fath-1* RNAi leads to viable worms, albeit with shortened body lengths and lifespans, we hypothesized that we would be able to recover heterozygous modified worms without affecting viability. Using ClinVar, pathogenic mutations were identified in a conserved proline early in the human gene. Three different groups of students each attempted to make a different mutation (a point mutation, a frameshift or a nonsense mutation) via short-range homology-directed repair *dpy-10(gf)* co-CRISPR. Each group used the same guide RNA but different single-stranded oligonucleotide donors. Each desired modification would disrupt an NdeI restriction site. Students spent six weeks on the project. During the first week they made injection pads and needles, practiced picking worms and familiarized themselves with the wild-type and roller phenotypes. During the next three weeks they injected worms for ~2 hours per group each week and screened their injected worms for rollers. During the last two weeks, students conducted molecular analysis of offspring worms, including the ProteinaseK treatment and PCR (week five) and the digest and agarose gel electrophoresis (week six). Although the class was not successful in making the modification, the students became reasonably proficient at injections by the end, and two students opted to continue the project in the following semester as an independent study. This exercise allowed students to learn about CRISPR and genetic engineering, model organism research, PCR, restriction digestion and gel electrophoresis while conducting original research.

1278B **Using *C. elegans* in course-based undergraduate research to tackle the amyloid-beta controversy** Jay Pieczynski Rollins College

Alzheimer's Disease (AD) is a major neurodegenerative disorder, leading to billions if not trillions of dollars in research and an untold number of working hours spent looking for molecular mechanisms and potential treatments. Aside from pharmaceutical research and development, there also exists an entire supplement industry around the prevention, delay, and treatment of AD. Many of these supplements have not been rigorously vetted by research, and if studies concerning supplements exist, their data is often used to mislead the consumer. One of the leading hypotheses concerning the mechanism of AD involves the formation and accumulation of amyloid plaques formed from aggregation of the amyloid beta (A-beta) protein in neurons. This A-beta

hypothesis has recently been called into question. There is a well-established *C. elegans* temperature sensitive model of A-beta aggregation that has been used repeatedly to assay the effects of A-beta accumulation. Using the A-beta *C. elegans* model, a semester long course-based undergraduate research experience (CURE) was built around the A-beta hypothesis and the surrounding controversy. First year undergraduate students used the *C. elegans* AD model to test six different natural organic products that are the active ingredient in supplements touted to treat or prevent AD. Students tested multiple behaviors to determine if any supplement had positive effects on worms overexpressing A-beta. In addition to learning the biological underpinnings of these assays, students also focused on the science skills of information literacy, critical thinking and analysis, rigor of peer review, and the iterative process of writing. From this experience, first students demonstrated increased confidence in these skills and more comfort in laboratory investigations.

1279B **Using *C. elegans* in a Cell Biology Course to Teach Core Concepts and Experimental Design** Janet UgolinoBiology, Monmouth College

Traditional cell biology courses often rely on "canned" labs designed to reinforce fundamental cell biology topics like cells structure, membrane transport, and metabolism. Although reinforcing these concepts is important, these labs often do not provide students with a "real-world" laboratory experience. Using a model organism like *C. elegans* in a cell biology course provides students with the opportunity to learn key cell biology concepts while at the same time participate in an authentic research experience. At Monmouth College, I have incorporated *C. elegans* into the laboratory component of our 200-level cell biology course to teach enzyme function, gene expression and regulation, and cell signaling. All laboratory exercises are inquiry-based and reinforce the concepts of experimental design and hypothesis testing. In the first module, the students learn how to work with the worm under the microscope and learn how researchers use knockout animals to study protein function by examining the thrashing behavior of an alcohol dehydrogenase (*sodh-1*) mutant exposed to ethanol. In the second module, students examine the expression of the glycerol 3-phosphate dehydrogenase (*gpdh-1*) gene in osmotic stress mutants using RT-PCR. In the third module, students study the cell signaling pathways responsible for volatile odor recognition use chemosensation assays. The majority of labs have a pre-lab exercise that requires students to craft a hypothesis and a post-lab "mini-manuscript" assignment that requires students to analyze, present, and interpret their data. In the last module, students are given the opportunity to use what they have learned during the semester to design and implement their own experiment using *C. elegans* and present their findings to their peers in the form of a poster presentation. The student response to these labs have been positive and has provided several of our majors with the skills necessary complete their senior research projects. Future work will involve incorporating *C. elegans* into our 100-level introductory biology courses and upper level neurobiology courses.

1280B **Optimizing *Caenorhabditis elegans*-based CUREs to screen for microbial natural products** Emily Washeleski, Christian Holmstrom, Megan Guyer, Kenzie Fox, Ina Klasner, Frida Vasser, Christina Boody, Isaac Bigcraft, Paul D Goetsch Biological Sciences, Michigan Technological University

Microbial natural products remain the largest untapped resource for drug discovery. With an estimated diversity of 4,000-50,000 microbial species per gram of soil, the primary challenge is how to effectively screen for bacterial strains that harbor anything useful. Inspired by the discovery of the nematocidal drug avermectin from soil bacterial isolates, we implemented and optimized an introductory genetics Course-Based Undergraduate Research Experience (CURE) aimed to identify potential useful metabolites through direct feeding of *Caenorhabditis elegans*. We use *C. elegans* as an ideal platform to screen for bioactive metabolites in wild bacterial isolates because (1) *C. elegans'* natural bacterial diet offers a straightforward treatment method, and (2) the plethora of available genetic and transgenic strains offers a rich platform for demonstrating the use of genetic model systems for human health research for the participating students. For the first CURE cohort, we demonstrated the feasibility of the CURE by assessing for suppression of the *let-60/*Ras oncogenic signaling pathway. One *Bacillus* species isolate inhibited the overactive *let-60/*Ras phenotype in both Multivulval (Muv) *let-60* worms and Synthetic Multivulval (SynMuv) *lin-52; lin-8* worms. Subsequent experiments identified that the *Bacillus* species causes developmental delay and growth suppression in wild-type N2 worms, as compared to OP50 *E. coli* control. In the process of characterizing the *Bacillus* species feeding effect, we developed two primary counter-screens to assess for false positives, including assessing for whether isolated microbial strains are nutritionally deficient through media supplementation or pathogenic through the *clec-60::GFP* reporter that expresses when the *C. elegans* immune response is activated. For the second CURE cohort, we attempted to screen through microbial consortia to streamline the bacterial isolate phase. We discovered that feeding worms in liquid culture a mixture of unknown, un-isolated bacteria led primarily to worm death. Finally, for the third CURE cohort, we focused primarily on optimizing the microbial isolation phase of the pipeline by assessing for diversity of isolated microbes following growth on three different nutritionally rich agar plates. Overall, our pipeline shows promise as both an accessible introductory research experience for life science trainees and an expandable screening method to identify bioactive natural products.

1281B **Nematode Hunters: an integrated approach combining science outreach, course-based undergraduate research, and mentored research to identify novel nematode viruses** Katherine Przeworski[1], India Cannon[2], Sehrish Khan[2], Lisa van Sluijs[3],

Chika Fujii[4], David Wang[4], Jessica Sowa[21]Pennsylvania Leadership Charter School, [2]Biology, West Chester University of Pennsylvania, [3]Wageningen University, [4]Washington University School of Medicine in St. Louis

C. elegans and other related free-living nematodes are a popular and powerful model system for studying the cell biology and evolution of host-pathogen interactions. However, at this time only four viruses capable of naturally infecting *Caenorhabditis* nematodes have been identified, and of those only one (Orsay virus) infects *C. elegans* (Félix et al., 2011; Frézal et al., 2019). This severely limits *C. elegans* as a model system for studying host-virus interactions specifically.

To search for novel nematode viruses capable of infecting *C. elegans*, we have taken a citizen-science approach to obtaining a diverse sampling of wild-caught nematodes. The Nematode Hunters outreach program partners with 4th grade classrooms in Pennsylvania, allowing teachers to implement a week-long classroom module. In this module, 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. Data collected 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.

Once received, samples are screened via co-culturing of wild nematodes with *C. elegans* expressing intracellular infection reporters. This screening is conducted by students enrolled in a course-based undergraduate research class which runs every fall. Using this approach we have evaluated over 400 wild nematode isolates sent in from both the Nematode Hunters program and other collaborators, identifying more than 200 potential intracellular infections. To prioritize potential viral infections, students in the course homogenized reporter-activated populations and passed the homogenate through a 0.22uM filter to size-exclude other pathogen types. Follow up testing is then conducted by students in the Sowa lab, including FISH staining to detect Orsay or other known nematode viruses and collection of samples for RNA sequencing. We have thus far identified and confirmed via sequencing two new variants of the Orsay virus, both discovered in samples originating in the Netherlands. We are continuing to test additional filtrate samples, now prioritizing samples collected from Pennsylvania.

1282C **Using wormPOP to broaden access to hypothesis-driven research.** Andrea ScharfBiological Sciences, Missouri University of Science and Technology

Access to research opportunities and hypothesis-driven projects is important for science education, science literacy, and to engage the next generation of scientist. However, many students for example in underserved communities or due to physical disabilities, have not access to any research opportunities. To reach our goal to diversify the STEM workforce we need to develop strategies to reduce barriers and provide more accessible hypothesis-driven research opportunities.

We developed wormPOP, an agent-based model, to study life-history traits and their impact on population dynamics[1]. wormPOP is a realistic computational simulation of an experimental population system with *C. elegans* that allows us to track and manipulate populations over many generations. Currently, we are working on a second educational version with an improved user-friendly interface and additional feature for visual outputs. The goal is to turn wormPOP into an educational tool for high schools and colleges that can be used in combination with laboratory *C. elegans* experiments or isolated in school settings with limited resources. In addition, it will be easily adjustable to different age-groups and experience levels.

1. Scharf, A., Mitteldorf, J., Armstead, B., Schneider, D., Jin, H., Kocsisova, Z., Tan, C., Sanchez, F., Brady, B., Ram, N., DiAntonio, G.B., Wilson, A.M., and Kornfeld, K. A laboratory and simulation platform to integrate individual life history traits and population dynamics. Nat Comput Sci 2, 90–101 (2022). https://doi.org/10.1038/s43588-022-00190-8

1283C **Using *C. elegans* to investigate the effect of hormones on fertility in a non-majors course** Priscilla Van Wynsberghe, Nancy Schult Biology, Colgate University

Hands-on laboratory experiences provide important ways to reinforce scientific concepts. Even the importance of fundamental experimental practices, like repetitions and the inclusion of controls, are better understood after analyzing data collected from one's own experiment. To help students better understand these and other ideas central to the practice of science, we have developed a simple, hands-on laboratory experiment that investigates the effects of hormones and hormone-related treatments on *C. elegans* fertility. We have implemented this experiment in two different non-major science courses entitled Hormones and The Biology of Women. The importance and functions of hormones in biological systems is a key component of both courses. Thus, inclusion of this experiment reinforces core course concepts while helping students understand how scientific knowledge and understanding are created, tested, and modified. In this experiment individual *C. elegans* are grown in the presence of different hormones, hormone-like molecules, or hormone-related treatments for one generation. Then, within a single class period, students count the number of progeny per worm. Class data is pooled and students are asked to analyze a subset of data

prior to the subsequent class period. Time is reserved in this second class for students to peer review each other's preliminary figures of a subset of the class data. This step is an important moment for students to gain experience in data analysis and presentation. Ultimately students describe their findings in a short lab report. We have found that though simple, this exercise is particularly powerful in helping students understand the importance of controls for comparisons, the importance of repetitions for confidence in conclusions, and that science takes time and careful attention to detail. In addition, students, many of whom will never take a laboratory-based science course, enjoy spending time in the lab observing these organisms.

1284C **A CURE for the Teaching Blues: A Collectivist Framework to Move a Research Project from Conception to Manuscript in a Single Semester of an Undergraduate Laboratory Course** Sara Olson, Louie KulberMolecular Biology, Pomona College

Investigative course-based undergraduate research experiences (CUREs) are known to promote the engagement and retention of students in STEM disciplines. They are also more exciting for instructors to teach than traditional cookbook labs that do not expose students to the authentic nature of science. For a recent CURE lab, I challenged students in my Advanced Cell Biology class to engage collectively with the full cycle of scientific inquiry in a single semester – to start with a new project idea, generate preliminary data, propose a mechanistic model, refine the model through iterative rounds of experimentation, and draft a full-length manuscript. I chose the project based on an exciting observation made by a previous Advanced Cell lab group – that a subset of proteins required for *C. elegans* eggshell formation appears to be secreted *independently* of the conventional COPII-dependent ER-to-Golgi trafficking pathway. The current students began by repeating the original experiments, which reinforced the importance of reproducibility in science, and allowed them to learn the standard cell biological techniques typically taught in the course. The students then shared their preliminary data and collectively developed a model to explain their findings, outlining a potential storyline for a manuscript. Next, they designed experiments to test possible explanations for the unexpected findings and to fill any gaps in knowledge that would allow them to complete the storyline, and spent the remainder of the semester conducting those experiments. At the end of the semester, students shared their findings in formal oral presentations and posters, and convened to discuss how to put the manuscript together and divide the work equitably among the lab groups. While we originally sought to complete a full manuscript in a single semester, we fell a bit shy of this ambitious goal. However, the paper is ~2/3 complete, and one student from the course joined our lab to complete the remaining experiments. Exit surveys showed the students were highly engaged with the lab, felt ownership over their project, became more confident in their ability to carry out scientific research, and came to value the collaborative and collective nature of research. This pedagogical experiment shows that significant progress toward a full-length publication can be made by undergraduates in a single semester. This model could help instructors at primarily undergraduate institutions (PUIs) improve research productivity during the busy academic year, and increase publication rates and student authorships.

1285C **Show not tell: Communicating the practical utility of C. elegans research** David Weinkove[1,2]1Department of Biosciences, Durham University, [2]Magnitude Biosciences Ltd

The size of the International Worm Meeting is a testament to the success of *C. elegans* as a model organism. Countless breakthrough discoveries including three Nobel prizes have demonstrated the power of the system to understand basic biology. However, all *C. elegans* scientists have to respond to numerous questions about translatability to human biology and whether public funding is justified for research on a nematode worm. *C. elegans* scientists are armed with a battery of standard responses to these questions, which usually involve pointing out homology at various levels from genes to organs or behaviours. Some of these claims are overstated and that can make them counterproductive. The best way to convince people that *C. elegans* research is useful is to show them examples of where *C. elegans* research has resulted in a new therapy or product that brings value to people. One great example is the work led by Ethan Perlstein and his company, Perlara, in which *C. elegans* was used to identify a repurposed drug to successfully treat a child with a rare disease, and that drug is now in a Phase III clinical trial. There are several examples of other therapies, initially discovered using *C. elegans* that are now in clinical trials. In recent years, *C. elegans* has been used by more and more companies that are either developing new therapies or acting as contract research organisation to help biotech and pharma companies to do so. Thus, many more examples will be appearing over the next few years. It is important that these examples are collated, presented and made accessible in a way that helps the whole community. I will discuss how this goal may be achieved and the results best used to address the question of utility to support further research across the whole *C. elegans* community.

1286V **Creating a curated collection of *C. elegans* CRISPR gene edits via the Glow Worms course-based undergraduate research education (CURE) curriculum** Ryan Doonan[1], Daniel J Dickinson[2]1Glow Worms, The University of Texas at Austin, [2]Molecular Biosciences, The University of Texas at Austin

CRISPR/Cas9 has recently revolutionized genomic engineering, allowing custom editing of endogenous genes in cells, tissues, and organisms. *C. elegans* is currently the multicellular model organism most amenable to CRISPR knock-in. Despite this amenability, there is a critical unmet need within the research community to develop strains carrying endogenous fluorescent protein tags,

replacing outdated GFP transgenes as pathway-specific, subcellular, cellular, or tissue-specific markers. To address this need, we have developed a course-based undergraduate research education (CURE) curriculum called “Glow Worms”. Glow Worms is an innovative infrastructure for offering undergraduates an immersion research experience while building a large collection of publicly available CRISPR-edited strains. The Freshman Research Initiative (FRI) at The University of Texas at Austin is the largest undergraduate research curriculum in the nation, serving 1000+ students per year. The Glow Worms research “stream” within FRI has served nearly 150 students and created over 100 worm strains in just over 3 years. Importantly, our community impact will be quantifiable based on how many research laboratories are utilizing our strains and how many publications are generated as a result. We estimate that if the goals of the stream are met, our strains will be utilized by hundreds of research laboratories over the next decade.

1287V **Implementation of a multi-year pre-collegiate biomedical, engineering, and environmental science research program** Ramon Herrera, Benjamin Holt, Louie Elliott, Mary Loveless, Elizabeth Burnette Baylor School

Baylor Research is a pre-collegiate research program that includes Biomedical, Engineering, and Environmental topics. The mission of Baylor Research is to teach students to think like scientists and engineers through cutting-edge research projects taught by experts in those fields. This program was established in 2016 and has grown to support 68 research students within the curriculum in the 22-23 school year. Five science electives (Engineering Design, Molecular Methods, Research I, Advanced Research, and Thesis Research) have been developed to support breadth and depth in these topic areas. Core research concepts and projects have also been implemented in the main curricular courses as well as Advanced Placement science courses. This work presents the infrastructure and methodology for successfully incorporating basic science research topics and techniques in a high school classroom. Additionally, college preparedness, matriculation into STEM-related college fields, and entry into research intensive program is discussed.